THE RELATION OF THE SPLEEN TO BLOOD DESTRUCTION AND REGENERATION AND TO HEMOLYTIC JAUNDICE.

XII. The Importance in the Production of Hemolytic Jaundice of the Path of Hemoglobin to the Liver.

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In connection with our studies (1) on the influence of splenectomy in diminishing the tendency to jaundice after the administration of hemolytic agents, our attention has been called to the possible importance of a factor purely mechanical; namely, the relation of the spleen to the blood supply of the liver.

Ponfick (2) in a discussion of hemoglobinemia of whatever origin and its consequences called attention to certain facts. He recalls that the earliest observers had already recognized that when hemolysis is going on the spleen becomes greatly swollen in consequence of the accumulation of disintegrating erythrocytes; for this swollen spleen Ponfick uses the term spodogenous (σποδός, waste products). Ponfick notes that simultaneously the liver eliminates a bile very rich in pigments and suggests that this is derived from the hemoglobin set free in the spleen, carried by the portal circulation to the liver and removed by this organ. Ponfick further expressed the view, based on experiments not quoted in detail, that the liver could completely remove and transform into bile pigment liberated hemoglobin up to the extent of one-sixtieth of the total hemoglobin of the body, but that hemoglobin set free in excess of this amount passes through the liver and is eliminated by the kidneys, causing hemoglobinuria. One-sixtieth of the total hemoglobin in the dog is about 0.18 gm. per kilo. In an earlier paper of this series, Pearce, Austin, and Eisenbrey (3) have shown that the injection of from 0.14 to 0.35 gm. per kilo of hemoglobin as laked blood will cause the appearance of hemoglobinuria, but that a factor of great importance, apparently overlooked by Ponfick, is the rate at which the hemoglobin is liberated in the circulation. The more slowly it is introduced the larger is the quantity that the liver can take up without permitting the concentration in the blood to reach at any time that required for the production of hemoglobinuria. It was, further shown in the same paper that while small amounts of injected hemoglobin are removed by the liver and presumably excreted as bile pigments

in the bile without the occurrence of jaundice, if the injected hemoglobin be in excess of 0.30 to 0.40 gm. per kilo, the liver is unable to eliminate all the bile pigment formed from the excess of hemoglobin and some of the bile pigment is resorbed from the liver and under these circumstances appears in the urine. It was noted in this respect also that the rate of injection is of great importance in determining the amount of hemoglobin that the liver will tolerate without the appearance of bile pigments in the urine. Very slow but long continued liberation of hemoglobin can eventually overtax the hepatic excretory power and lead to the appearance of bile pigments in the urine, although the hemoglobin liberation may have been slow enough to permit of its continued adequate removal from the circulation by the liver with at no time the development of hemoglobinuria. Thus the first effect of hemoglobin liberation into the blood is an increased bile pigment content of the bile. This was shown experimentally by Tarchanoff (4). If the amount of hemoglobin be small enough and its liberation slow enough this is the only effect. A slightly larger amount rapidly liberated will produce hemoglobinuria. A larger amount extremely slowly liberated will produce bile in the urine. A larger amount liberated at an intermediate rate may produce both hemoglobinuria and bile pigments in the urine. Ponfick in his experiments noted bile pigments in the urine but follows Tarchanoff's explanation and attributes them to the now generally repudiated theory of extrahepatic conversion of hemoglobin into bile pigment.

Following Ponfick many other workers have attributed importance to the spleen as the site of disintegration of erythrocytes; among these may be mentioned Hunter (5), Gabbi (6), and Mya (7). Bottazzi (8) in his studies of the blood after splenectomy noted an increased resistance of the erythrocytes to hypotonic salt solutions and it was to this factor that Banti (9) attributed the greater resistance and diminished tendency to jaundice of splenectomized animals receiving hemolytic agents.

However, Pugliese and Luzzatti (10) failed to confirm Bottazzi's findings, but, noting with Banti and others the diminished tendency to jaundice after splenectomy, they made further studies along the lines suggested by Ponfick's observations and elaborated the following hypothesis. The spleen is the natural location for the disintegration of erythrocytes after the administration of hemolytic poisons, and the hemoglobin so liberated is carried directly by the portal system to the liver, there at least in large measure to be removed, converted into bile pigment, and excreted in the bile; or, if present in great quantity, to be resorbed and appear in the urine and tissues as bile pigments and thus produce jaundice. On the other hand, in the absence of the spleen, the blood cells undergo disintegration elsewhere, probably chiefly in the bone marrow, as noted by Martinotti and Barbacci (11). Hemoglobin liberated in the bone marrow could under these circumstances reach the liver only through the general circulation. It would therefore be diluted and moreover would reach the liver largely through the hepatic artery,—a vessel normally carrying blood for nutritive purposes, not for purposes of elaboration. They further note that the slow flow of blood through the bone marrow might delay the removal of the liberated hemoglobin. For these reasons in the splenectomized animal it is to be expected that the hemoglobin would reach the liver much more gradually and at a rate, indeed, which might well lie within the capacity of the liver for complete excre-

tion as bile pigment; hence no reabsorption of bile pigments would occur and jaundice would not develop.

These authors were able to show by the aid of a bile fistula that while the other constituents of the bile are but little altered by splenectomy, the bile pigments are reduced to about one-half. Moreover, after the administration of hemolytic poisons while the bile of the splenectomized animal shows an increase in the bile pigments, this is not so pronounced as in the normal animal after hemolytic poisons; the increased pigmentation of the bile is, however, of longer duration in the splenectomized animal.

It may be noted in passing that these observers failed to find disintegrating erythrocytes in the lymph nodes after splenectomy; a phenomenon which Pearce and Austin (12) found conspicuous; nor do the findings of Karsner and Pearce (13) regarding the fragility of the corpuscles after splenectomy confirm those of Pugliese and Luzzatti, but on the contrary agree with those of Bottazzi, to the effect that there is an increased resistance of the erythrocytes to hypotonic salt solutions and to hemolytic immune serum after splenectomy.

In order to test the hypothesis of Pugliese and Luzzatti, we have injected a constant amount per kilo of hemoglobin solution in the form of laked blood into a series of dogs, injecting each at least twice, once into the general circulation by way of the femoral vein and once into the portal circulation by way of a mesenteric vein. The rate of injection has been always the same. In some instances, the femoral injection was given first, in other instances the mesenteric. At least five days were allowed to elapse between injections. We have thus been able to study the effect of the site of the injection upon the development of hemoglobinuria and of bile pigments in the urine, and from our results believe we may draw conclusions as to the fate of hemoglobin when liberated into the portal system, on the one hand, or into the general circulation, on the other.

In these experiments, a fasting, normal dog was bled, the blood defibrinated, the cells thrown down by centrifuging, and the supernatant serum removed. About four volumes of distilled water were then added to the cells and the mixture agitated for fifteen to twenty minutes to induce hemolysis. The solution was then centrifuged rapidly for twenty minutes to remove most of the cell stromata, was made isotonic by addition of sodium chloride, and centrifuged to remove any globulin thrown out of solution upon adding the salt. One cc. of this solution was then diluted with 99 cc. of distilled water and its hemoglobin strength determined in

a Fleischl-Miescher hemoglobinometer. As much of this solution as should equal either 0.3 gm. or 0.4 gm. of hemoglobin per kilo of body weight was then injected intravenously into a normal dog. Injections were given at such a rate that the entire injection should occupy one minute per kilo of body weight. All bleedings and injections were made under ether anesthesia. Injections into the general circulation were made into one of the small veins of the leg. Portal injections were made by drawing a loop of intestine from the abdomen under aseptic precautions, and injecting through a needle into a small mesenteric vein. In some instances water was given by stomach tube at the close of the operation. The urine was then collected, the dog being kept in a metabolism cage, and if hemoglobin appeared the amount was estimated either directly in the Fleischl-Miescher hemoglobinometer or by comparison with a standardized acid hematin solution. In addition, we followed the jaundice by the persistence of bile pigments in the urine after hemoglobin injection into either the mesenteric or the femoral vein. The urine was examined for bile pigments by the Rosenbach test.

TABLE I.

Influence of Site of Injection on Amount of Hemoglobin Eliminated in the Urine.

Hemoglobin injections.	Date.	Gm. of hemoglobin per kilo eliminated after injection into	
		Femoral vein.	Mesenteric vein.
Dog 26 (0.4 gm. per kilo).....	Mar. 20	0.085	
	" 26		0.043
" 12 (0.3 " " ").....	Feb. 25		0.029
	Apr. 15	0.043	
" 5 (0.3 " " ").....	Jan. 9	0.024	
	" 15		None.
	Mar. 2		"
	" 26	0.026	
" 3 (0.3 " " ").....	Jan. 7	0.017	
	" 15		Trace.
Splenectomized.....	Feb. 19		
	Mar. 2		None.
	" 20	0.025	
" 49 (0.3 gm. per kilo).....	Feb. 25	0.014	
Three months after splenectomy..	Apr. 15		0.010

The results as regards hemoglobinuria are shown in Table I. In each of five dogs used, the output of hemoglobin by the kidney was much less when the hemoglobin was introduced into the mesenteric

vein than when introduced into the femoral vein, and this is true regardless of which injection was performed first. This we attribute to the removal of the hemoglobin to a greater extent by the liver when the injection is made into a mesenteric vein with the result that the hemoglobin reaching the general circulation is less concentrated and is less likely to be eliminated by the kidneys and appear in the urine.

TABLE II.

Persistence of Bile Pigment in the Urine after Hemoglobin Injection as Determined by Point of Injection.

Hemoglobin injections.	Date.	Persistence after injection into	
		Femoral vein.	Mesenteric vein.
		days	*days*
Dog 4 (0.3 gm. per kilo).....	Jan. 9	4	
	" 16	4	
" 5 (0.3 " " ").....	" 9	3	
	" 15		12+
" 25 (0.4 " " ").....	Mar. 21	4	
	" 26		7+
" 26 (0.4 " " ").....	" 20	4	
	" 26		7+
" 12 (0.3 " " ").....	Feb. 25		5
	Apr. 15	4	
" 3 (0.3 " " ").....	Jan. 7	0	
	" 15		9
Splenectomized	Feb. 19		
	Mar. 2		4
	" 20	0	
" 49 (0.3 gm. per kilo).....	Feb. 25	4	
Three months after splenectomy..	Apr. 15		6

The results of the study of the degree and persistence of jaundice (as indicated by bile pigments in the urine) in the dogs after the two types of injection are shown in Table II. It will be seen that in six dogs studied the jaundice was distinctly more persistent after mesenteric than after femoral injection, and this was true regardless of which injection was made first. In Dog 4, two successive injections were made into the femoral vein to determine whether the second injection would give a result notably different from the first. Such was not the case, the duration of the bile pigments being the same after each injection when both were made into the femoral vein.

In our studies both of hemoglobinuria and of the persistence of

jaundice after hemoglobin injections we have employed splenectomized as well as normal dogs, but have found that splenectomy has no influence upon the fate of hemoglobin injected into either the general or portal circulation.

SUMMARY.

These experiments indicate, therefore, that when hemoglobin is set free in the portal circulation a larger amount is held by the liver and converted rapidly into bile pigment than is the case when it is set free in the general circulation, and that, under the former condition, over-loading of the liver with bile pigment more readily occurs and jaundice is more apt to develop.

This mechanical influence must, therefore, be a factor in the lessened tendency after splenctomy to the jaundice which follows blood destruction due to hemolytic agents, for whether the spleen be an active factor in destroying the erythrocytes or whether it plays merely a passive part as a place for the deposition of the disintegrating cells, there can be no question that in this organ, when it is present, a large number of cells undergo their final disintegration after the action of hemolytic poisons, and that the hemoglobin there liberated passes by the portal system directly to the liver. When the spleen is removed, this disintegration occurs in other organs, notably in the lymph nodes and bone marrow, and the hemoglobin from these organs passes not into the portal but into the general circulation, from which it reaches the liver more gradually and in a more dilute form.

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THE RELATION OF THE SPLEEN TO BLOOD DESTRUCTION AND REGENERATION AND TO HEMOLYTIC JAUNDICE.

XIII. The Influence of Diet upon the Anemia Following Splenectomy.

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In our various experimental studies (1) of the changes in the blood that follow, in the dog, the removal of the spleen, we have been impressed by the wide variation in the degree of anemia. In the severer forms of anemia the changes are marked, as, for example, a drop in red cells from 5,100,000 to 2,970,000 and in hemoglobin from 105 to 50 per cent in five weeks; in a milder form, the red cells may not fall below 4,000,000 to 4,500,000, with a decrease in hemoglobin to 65 or 75 per cent. Most of the animals show changes corresponding roughly to one or the other of these types. There remains, however, a relatively small group in which the changes are very slight, representing a decrease of only about 1,000,000 red cells and only 10 to 15 per cent of hemoglobin. These slighter changes, falling sometimes within the limit of error of the methods of blood counting, led us to ask ourselves the question: May there not be some factor other than the absence of the spleen that aids in producing the severer forms of anemia? That the absence of the spleen is the main factor there can be no doubt, for even in the milder forms the slight changes usually correspond to the period (three to six weeks) in which the severer anemias reached their lowest point; also, animals with the milder forms show the same tendency to slow repair when a hemolytic agent is given, as do the severer forms. There is, therefore, no

doubt about the removal of the spleen being responsible for a disturbance of the mechanism concerned in maintaining the normal blood picture. The question remains, however: Why does the degree of anemia vary? Our first thought was that diet might have some influence. Until the present investigation was undertaken, all animals, except those used in the study of iron metabolism (2), had been kept upon the same general diet; *i. e.*, a mixture of meat, bread, cereals, and vegetables, in all essentials the table scraps upon which dogs are usually fed. This was always supplied in abundance and each dog received all he would eat, and, as our records show that dogs splenectomized for periods of several months or a year or more gained in weight on this diet, we considered it perfectly satisfactory. However, we did not know the exact caloric value of this mixed diet and, moreover, as it was essentially a boiled diet, it might possibly be deficient in some substance essential to the proper function of the hemopoietic system.

In this connection we recalled (1) the observations of Asher and Vogel (3), that while an iron-poor diet (sugar, starch, and lard) has no effect upon the blood picture in a normal dog, in the splenectomized dog on the same diet a great decrease in number of red cells and amount of hemoglobin occurs, and further, that if under the latter circumstances an iron-rich (flesh) diet is given, the blood picture quickly returns to normal; (2) Richet's (4) observation that in order to maintain splenectomized dogs at the same weight as normal dogs, a much larger quantity of food is necessary; and (3) Paton's (5) conclusion that splenectomy in the dog has no influence either upon the blood picture or the general metabolism.

The various observations quoted, with the exception of Paton's studies of metabolism, are directly opposed to the results of our studies. In regard to Asher and Vogel's contention, we have not found by a direct quantitative study of the elimination of iron that splenectomy seriously influences iron metabolism (6). Moreover, the improvement in the anemia which they describe as the result of feeding iron-rich food corresponds in our opinion to the spontaneous repair of the anemia which usually begins about the end of the fourth week. In other words, the improvement they noticed was in part at least due, in our opinion, to the normal repair and not to the effect of the iron-rich food. Their conclusions would be more convincing if they had prevented entirely or lessened the severity of the anemia by beginning the feeding before or immediately after splenectomy instead of waiting nearly three weeks. This phase of the problem we have investigated in our present study. Richet's point, as also Paton's observations, demands carefully conducted metabolism studies and these, carried out simultaneously with our dietary studies, are presented in a separate communication (7). Here, however, it may be noted that in the normal animal we have found that the removal of the spleen has no influence upon metabolism. Moreover, we have not seen noteworthy changes in the weight of our splenectomized animals. For

a few days after splenectomy, a slight loss may occur, but in all long time experiments an increase in weight has been observed.

The studies of Paton and his associates as to the changes in the blood after splenectomy are the most carefully conducted of any in the literature and for this reason we have been greatly disturbed that our results were so different. Their studies, however, were limited to two splenectomized animals and two controls and it may be that by chance the former correspond to the milder anemias which we observed. In this connection it is of interest to note that in the splenectomized dog they did obtain a decrease of 600,000 to 800,000 red cells, which, however, as it occurred also in a normal dog was not, by contrast, of apparent importance. Moreover, they used puppies, about two and a half months old, and it is possible that in such young animals the mechanism of blood regeneration may be more active than it is in older animals. Our observations were all upon full grown animals. In Paton's blood work diet is not mentioned, but in his metabolism work the dogs were, for part of the time at least, on a meat (high iron) diet, which, if used in the blood work also, might have been, if Asher and Vogel are correct, a factor in decreasing the anemia. Thus it is evident from our own experience and from this brief review of the discordant conclusions in the literature of the subject that information of value might be obtained from carefully controlled dietary studies. Such studies we present at this time.

In our first group of experiments, animals were placed on caloric-ally sufficient diets, the protein being furnished in the form of beef heart, beef spleen, or commercial casein, and the fat and carbohydrate in the form of lard and bread crumbs. Beef spleen was introduced on account of its large iron content, in contrast with that

TABLE I.

Days.	Raw beef heart, lard, and bread.						Days.	Raw beef heart, lard, and bread.		
	Dog 79 (splenectomized).			Dog 83 (splenectomized).				Dog 81 (control).		
	Weight.	Red cell count.	Hemoglobin.	Weight.	Red cell count.	Hemoglobin.		Weight.	Red cell count.	Hemoglobin.
Before splenectomy								*kilos*		*per cent*
							1	9.1	6,320,000	97
	kilos		*per cent*	*kilos*		*per cent*	4– 7		6,650,000	102
	9.3	7,930,000	110	8.7	7,000,000	107	12–18		6,880,000	96
		7,560,000	114		7,770,000	105	26–40	10.0	6,800,000	90
							40–60	10.1	6,000,000	96
After splenectomy										
5– 7	8.8	7,420,000	97	8.6	7,910,000	105				
10–14		7,230,000	98		7,720,000	103				
18–23		6,250,000	98	8.6	6,500,000	96				
26–33		6,810,000	96	8.7	7,270,000	101				
38–40	9.8	7,360,000	104	8.3	6,880,000	97				
45–48	10.5	6,690,000	100	8.5	6,260,000	96				
52–61	10.8	6,640,000	95	8.6	6,240,000	93				

of the beef heart and the casein. Several blood examinations were made during a period of ten days to two weeks before splenectomy and at intervals seldom exceeding a week after operation. All operations were done under ether anesthesia and for most of these we are indebted to Dr. Max M. Peet of the Department of Surgical Research. The animals were kept under absolutely uniform conditions. In Tables I, II, and III, which show the results of these

TABLE II.

Days.	Casein, lard, and bread. Dog 82 (splenectomized). Weight	Red cell count.	Hemoglobin.	Dog 84 (splenectomized). Weight.	Red cell count.	Hemoglobin.	Days.	Casein, lard, and bread. Dog 80 (control). Weight.	Red cell count.	Hemoglobin.
Before splenectomy										
	kilos		*per cent*	*kilos*		*per cent*	1	*kilos* 9.1	7,860,000	*per cent* 90
	16.7	5,500,000	98	8.7	7,780,000	98	4– 7		7,420,000	92
		5,551,000	85		7,710,000	97	12–18		7,020,000	96
							26–40	8.3	7,370,000	96
							40–60	9.2	7,180,000	103
After splenectomy										
5– 7	16.5	4,600,000	75	8.6	7,090,000	89				
10–14	17.2	4,688,000	65	8.8	7,140,000	92				
18–23		4,210,000	76	8.9	6,600,000	91				
26–33		5,160,000	82	9.1	7,010,000	92				
38–40		6,040,000	88	9.2	6,940,000	87				
45–48					6,630,000	91				
52–61	16.9	6,060,000	90	9.1						

studies, only the last two blood counts of the preliminary periods are given. These represent, usually, counts made respectively 1 to 2 and 5 to 7 days before splenectomy. As the blood of the several dogs was not always examined at exactly the same intervals after splenectomy, in order to shorten the table only enough blood counts are given to show the general trend of the blood picture. The estimation of iron and of nitrogen in the diet is based on the average of several estimations of the food materials used. These figures with a calculation of the caloric value of the food are given in Table IV.

Discussion of Tables I, II, and III.

By comparing the Tables I, II, and III it is at once evident that in no instance did the general nutrition of the animal suffer. A slight loss of weight occurred after operation, but this was soon re-

TABLE III.

Days.	Raw beef spleen, lard, and bread. Dog 85 (splenectomized). Weight.	Red cell count.	Hemoglobin.	Dog 86 (splenectomized). Weight.	Red cell count.	Hemoglobin.	Dog 87 (splenectomized). Weight.	Red cell count.	Hemoglobin.	Days.	Raw beef spleen, lard, and bread. Dog 90 (control). Weight.	Red cell count.	Hemoglobin.
Before splenectomy													
	kilos		*per cent*	*kilos*		*per cent*	*kilos*		*per cent*	1	*kilos* 7.2	5,800,000	*per cent* 92
	6.7	6,200,000	88	12.2	5,330,000	95	10.1	6,580,000	105	4–7		5,940,000	94
					5,450,000	95		5,900,000	100	12–18		5,680,000	92
After splenectomy											Discontinued because of development of distemper.		
5–7	6.5	5,880,000	78	11.5	4,360,000	76	9.4	6,490,000	108				
10–14		4,720,000	75		4,780,000	85		6,250,000	98				
18–23		4,850,000	80		5,040,000	82		6,080,000	96				
26–33		4,820,000	80		5,170,000	85		6,360,000	100				
38–40	6.9	5,180,000	82	12.8	5,800,000	94	9.9	6,410,000	101				
45–48		5,740,000	82	12.3	5,820,000	96		5,820,000	96				
52–61	6.9	6,040,000	94				10.0	5,730,000	102				

TABLE IV.

Nitrogen and Iron Content and Caloric Value of Diets of Tables I, II, and III.

Dog No.	Actual total per day.			Per kilo of body weight per day.		
	Nitrogen.	Iron.	Calories.	Nitrogen.	Iron.	Calories.
	gm.	*mg.*		*gm.*	*mg.*	
79	6.5	11.8	709	0.72	1.31	79
83	9.8	18.0	1,043	1.17	2.15	124
81	6.5	11.8	665	0.68	1.23	69
82	13.6	11.3	1,194	0.81	0.68	71
84	10.6	9.1	956	1.18	1.01	106
80	7.4	6.2	664	0.82	0.69	74
85	4.6	352.0	517	0.69	53.00	77
86	8.3	653.4	893	0.69	54.00	74
87	6.8	543.0	752	0.68	54.00	75
90	4.6	352.0	517	0.64	49.00	72

gained. Also it is seen that in no instance does a splenectomized dog maintain the same constant level of red cell and hemoglobin content as do the non-splenectomized animals. The change, however, in Dogs 79, 83, 84, and 87 is so slight as to be within the limit of error of the methods of blood examination; in Dogs 82, 85, and 86 the change is more marked, but even here one can hardly refer to the condition present as a frank anemia. It is, however, of significance that in all instances the variations are more marked than in the controls and also that they usually occur after about four weeks, the period, in severe splenectomy anemia, marked by the lowest counts. On the other hand, the question arises: Are these favorable results due to the diet, that is, to the general character of the diet or to the presence in the animals on a meat diet of large amounts of iron? That iron in the diet is a factor seems doubtful in view of the fact that two of the three animals (85 and 86) fed with beef spleen showed the most marked changes of any in the group. Beef spleen was selected because it contains a large amount of iron, according to our analyses 235 mg. per 100 grams, presumably in large part in organic combination and therefore readily utilizable. Fresh beef heart and casein, on the other hand, contain only 4.6 mg. and 7.2 mg. per 100 grams respectively, and if iron is an important factor in preventing anemia after splenectomy, one would not expect animals fed with spleen to show the changes evident in the figures given for Dogs 85 and 86; rather, one would expect figures as in

Dog 87. That the administration of abundant organic iron in the form of beef spleen did not prevent the anemia is in accord with our studies (8) of iron metabolism in the absence of the spleen and opposed to the conclusion of Asher and his associates.

On the other hand, in view of the slight changes which occurred in many of the animals, it is impossible to avoid the question as to whether a diet, adequate for the normal dog, is in some way inadequate for the splenectomized dog; if so, the value of our views concerning the severer types of anemia following splenectomy based on our earlier experiments upon dogs fed on a general mixed diet would depend upon whether or not the inadequacy of diet held for all animals operated upon, or only for animals without a spleen. If anemia occurred in dogs fed on the mixed diet after other operations than splenectomy, it would be at once evident that the food, while sufficient for a normal dog, was not sufficient for a convalescent dog. On the other hand, if the anemia could be demonstrated only after splenectomy, there would be established a point of importance in regard to the spleen in its relation to metabolism, and our observations on the anemia after splenectomy would not only be substantiated, but would gain an added importance. To settle this point, it was essential, therefore, to study in animals on our routine mixed diet the effect of splenectomy, and as a control some other simple operation involving the removal of an organ. Nephrectomy was selected as an operation quite analogous, from the technical point of view, to splenectomy and accordingly two healthy dogs were placed upon ordinary kennel diet for seventeen days; upon each dog a nephrectomy was then performed and the animal kept on the same diet for twenty-three days longer; splenectomy was then performed upon each dog and the animals kept on the same diet for thirty-eight days more. Blood counts were made at frequent intervals throughout the experiment. Whereas during the seventeen days on the diet before operation and during the twenty-three days following nephrectomy no significant change (Table V) in the hemoglobin or red blood cells was observed in either animal, after the splenectomy both showed a well marked fall in hemoglobin and red blood cell count. It is noteworthy also that relatively slight changes in weight occurred.

TABLE V.

Effect of Splenectomy Controlled by Previous Nephrectomy. Mixed Diet.

Date.	Dog 23.			Dog 42.		
	Weight.	Hemoglobin.	Red blood count.	Weight.	Hemoglobin.	Red blood count.
	kilos	*per cent*		*kilos*	*per cent*	
May 18..	10.9	100	6,840,000	14.1	102	7,810,000
" 25..	10.9	98	7,240,000	14.0	102	7,850,000
June 1..	11.0	92	6,760,000	14.9	101	8,330,000
" 4..	Nephrectomy			Nephrectomy		
" 8..	11.1	93	6,280,000	14.0	98	7,210,000
" 15..	11.3	90	6,020,000	14.6	95	7,120,000
" 25..	11.4	97	6,690,000	14.8	97	7,010,000
" 27..	Splenectomy			Splenectomy		
July 3..	10.9	90	6,600,000	15.1	90	6,840,000
" 10..	10.8	83	5,890,000	14.9	92	6,830,000
" 18..	10.8	77	5,740,000	14.9	88	6,480,000
" 27..	10.4	66	5,080,000	14.3	75	6,060,000
Aug. 4..	10.4	68	4,540,000	14.2	76	6,350,000

From these observations four conclusions may be drawn: (1) that inasmuch as the animals did not lose greatly in weight, the routine table scrap diet is a satisfactory food for animals after surgical operations; (2) that on this diet operation involving the removal of an organ other than the spleen does not cause anemia; (3) that the anemia following splenectomy is not to be explained, in view of the fact that the splenectomized animals maintained their average weight, by insufficient nutrition; and (4) if the anemia is in any way related to the diet it is either (a) because some toxic substance operative in the absence of the spleen is present in this particular food, or (b) because some substance present in the diet and normally utilizable can not be utilized in the absence of the spleen. In connection with this last conclusion it occurred to us that as the routine kennel diet is essentially a cooked diet it was possible that in the cooking there occurred the destruction by heat of some vitamine-like substance normally utilized by the spleen. To control this point a new series of observations were made (Table VI).

TABLE VI.

The Influence upon the Anemia Following Splenectomy of a Raw and a Cooked Diet.

Dog No.	Diet.	Before splenectomy.				After splenectomy.				Food values per kilo of body weight.	
		Period.	Weight before operation.	Hemoglobin.	Red cell count.	Period.	Final weight.	Hemoglobin.	Red cell count.	Nitrogen.	Calories.
		days	*kilos*	*per cent*		*weeks*	*kilos*	*per cent*		*gm.*	
48	Raw	28	13.4	99	5,450,000	6th– 9th	14.0	96	5,590,000	0.41	69
57	"	25	10.9	100	6,220,000	6th–12th	12.4	83	4,499,000	0.46	75
53	"	24	8.0	99	6,140,000	8th–12th	8.9	75	4,920,000	0.74	69
50	"	41	8.5	104	6,910,000	10th–13th	8.6	83	5,551,000	0.15	72
52	Cooked	48	10.8	105	6,760,000	7th– 9th	11.4	77	5,130,000	0.40	69
56*	"	121	8.4	88	6,250,000	6th–10th	7.8	61	4,880,000	0.40	73
Controls.											
9	Raw	108	11.4	102	6,620,000 (initial period)						
			12.9	95	6,590,000 (final period)						
56*	Cooked	121	8.2	100	6,460,000 (initial period)						
			8.4	88	6,250,000 (final period)						

* This animal was used first as a control for the cooked diet and later was splenectomized.

Six animals were placed upon a calorically sufficient diet, accurately determined; the only differences were that four received raw and two cooked meat. Examination of the blood was made at intervals of not longer than seven days. At the same time metabolism studies, the results of which are reported elsewhere (7), were made on some of the animals (Dogs 48, 52, 56, and 57). The diet in each of these experiments consisted of beef heart, lard, and sugar, a small amount of sodium chloride, and sufficient bone ash to ensure firm feces. Details as to nitrogen content and caloric value of the food are given in the table.

In connection with Table VI, it should be explained that, in order to place the figures covering all animals in one graphic table, the

counts given represent averages of several examinations. The figures before splenectomy represent the averages of the last three counts before operation; the figures after splenectomy the average of the three lowest consecutive counts. The figures for the two control animals represent the average of the first three and last three counts respectively.

It is evident from a study of Table VI that there is a greater tendency for animals on the cooked diet to develop anemia than is the case with those receiving raw meat. Thus in the latter group no change in the blood picture was evident in one animal while in the other three with moderate anemia, the hemoglobin did not fall below 75 or the red cells much below 5,000,000. On the other hand in the group receiving cooked meat both showed a marked change in the blood picture and in one a hemoglobin content as low as 61. As in all our previous studies the hemoglobin decrease is relatively greater than the fall in red cells. That the amount of protein given in the raw food is not an important matter is seen by contrasting Dog 53 on a high nitrogen diet with Dog 50 on a low nitrogen diet. In these two animals the calories of the diet were maintained by varying the amount of fat. The difference in the degree of anemia is negligible. In connection with the problem of the influence of cooked diet, it is noteworthy that Dog 56, which served as a control to Dog 52 for four months before it was splenectomized, and was for all this time on a cooked diet, showed during this time a falling off in the hemoglobin content of its blood. Moreover this animal was the only one showing a persistent loss of weight after splenectomy. Definite conclusions cannot be drawn from such a small number of experiments, but the fact that splenectomized animals on cooked beef develop an anemia of a degree more closely approaching that of animals on the usual kennel diet, which is essentially a cooked diet, while animals on a raw diet have a less severe anemia, suggests that heat destroys something in the diet which in the absence of the spleen tends to cause anemia. In view, however, of the relatively slight differences which we have found, experiments on a large number of animals on diverse diets must be made before a final decision is reached. Such observations are now in progress and we hope later to amplify this statement of the influence of diet.

SUMMARY.

The anemia which develops after splenectomy is most marked in animals on a mixed table scrap diet of meat, bread, cereals, and vegetables, which is essentially a cooked diet. Control studies in which a unilateral nephrectomy precedes splenectomy demonstrate that the anemia is not due to operation, hemorrhage, or accidents of convalescence but develops only in the absence of the spleen. The results of studies of the influence of food containing a large amount of iron in presumably easily utilizable form, as in raw beef spleen, do not support the view that the anemia is due to lack of iron in the food. Observation on the influence of a diet of raw meat as contrasted with cooked meat shows a more severe anemia in animals on the cooked diet and suggests the possibility that heat alters some substance which, in the absence of the spleen, the body cannot utilize. A final conclusion in regard to this point must, however, await the results of more detailed studies now in progress.

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STUDIES ON THE CIRCULATION IN MAN.

XVI. A Study of the Development of the Collateral Circulation in the Right Hand after Ligation of the Innominate Artery for Subclavian Aneurysm.

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Through the kindness of my colleague, Dr. Carl A. Hamann, I have been enabled to study two cases in which he successfully ligated the innominate and common carotid arteries for subclavian aneurysm. I have employed the method of measuring the blood flow in the hands previously described by me.[1]

The results in the first case, that of Mrs. K., 68 years of age, have already been published,[2] and need only be briefly alluded to here for comparison with the second case. I did not have the opportunity of examining the blood flow before the operation on Mrs. K. The operation was performed on Feb. 26, 1913. On Mar. 20, the flow in the right hand was 1.50 gm. per 100 cc. of hand per minute, and in the left 5.32 gm. (ratio 1 : 3.54), with room temperature 26.7° C. On Mar. 21 the flows were 1.83 gm. and 6.38 gm. for the right and left hands, respectively (ratio 1 : 3.48), with room temperature 22.7° C. On July 9, 1913 (19 weeks after the operation), the flow in the right hand was 8.26 gm. per 100 cc. per minute and in the left 10.69 gm. (ratio 1 : 1.3), with room temperature 26.2° C. There was no pulse in the accessible arteries of the right arm. Yet it is obvious from the blood flow measurements that a very satisfactory collateral circulation had been established. At the present time the patient is still alive, and a pulse has returned.

The second case was that of a colored man, Arthur B., aged 25 years, height 5 feet, 4 inches, weight 132 pounds. He was admitted to the City Hospital May 6, 1915, complaining of pain in the right shoulder and right arm. The pain began 3 weeks before he applied for admission coincidently with the appearance of a small lump below the right clavicle, which rapidly increased in size. The pain and muscular weakness in the right arm soon forced him to quit work. On admission the loss of power in the right forearm and hand was marked; in the upper arm and shoulder it was less marked, though evident. There is no atrophy

[1] Stewart, G. N., *Heart*, 1911, iii, 33.

[2] Stewart, *Arch. Int. Med.*, 1914, xiii, 1.

or edema of the right arm. The circumference of the right arm at the middle of the biceps is 30 cm., of the left 27 cm. The circumference of the right forearm is 28½ cm., of the left 27 cm. The man is right handed. The left radial pulse is of greater volume than the right. The pulse in the two radials is synchronous. Blood pressure in right arm, systolic 110, diastolic 88; in left arm, systolic 130, diastolic 70. The fingers of the right hand are markedly clubbed.

May 11, 1915. Blood: leucocytes 11,600, hemoglobin 80 per cent. Wassermann +++.

On May 11 the innominate and common carotid arteries were ligated.

May 19, 1915. He is fairly well. The right hand is not cold. He has no pain in the right arm, but the arm and hand feel tired. The radial side of the palm of the hand feels numb. There is no pulse at the left wrist. Pulse rate 116 (sitting). The blood flow in the hands was measured May 8, that is, 3 days before the operation, and again on May 22, 11 days after the operation. Further examinations were made on May 28, June 4, and June 11.

The patient's friends prevailed on him to leave the hospital on May 24, and he subsequently returned from time to time for the blood flow examinations.

On May 8 the flow in the right hand was 12.52 grams per 100 cc. of hand per minute for the last nine minutes in the calorimeters, and that in the left hand 6.36 grams, with average room temperature of 22.5° C. It may appear puzzling at first thought that the flow in the right hand should be double that in the left, while the amplitude of the right radial pulse is so much smaller than that of the left. The pulse as felt by the finger, however, is only a rough criterion of the blood flow on the assumption that the anatomical conditions are normal. In the present case the pulse wave must be supposed to be greatly diminished and its form distorted in passing through the aneurysm, but that is no reason for expecting that the mass movement of the blood should be diminished as well. The systolic pressure in the right arm was 110, the diastolic 88 mm. of mercury. The pulse pressure, which can alone be detected by the finger, is only 22 mm. of mercury. In the left arm the systolic pressure was 130, the diastolic 70, and the pulse pressure 60 mm. of mercury. But while this makes it clear that there is no ground for expecting a smaller flow on the side of the smaller pulse, why should the flow be so much larger on that side? The explanation is probably twofold: first, there is evidence of pressure on constituents of the brachial plexus supplying the right hand. Now pressure sufficient to cause loss of power in the skeletal muscles may be assumed to cause also some loss of vasomotor tone, since the vasomotor

fibers in the brachial plexus cannot conceivably be protected from the pressure. A loss of vasomotor tone in a hand will of course be accompanied by an increased blood flow. As a matter of fact, I have found that in early unilateral brachial neuritis the blood flow in the corresponding hand is decidedly greater than in the normal hand. Secondly, it is very likely that a dilated right subclavian artery offers a freer passage to the blood than the normal left subclavian does. That such reciprocal relations have an important influence on the distribution of the blood is indicated in an interesting manner by the results of the first blood flow examination after ligation of the innominate. On May 22 (eleven days after the operation) the flow in the right hand was 3.44 grams per 100 cc. per minute and that in the left hand 15.38 grams (ratio 1 : 4.47), with room temperature 25.0° C. The flow in the right hand has, of course, been greatly reduced by the ligation, but the interesting point is that the flow in the left hand has been correspondingly increased. Thus, 100 cc. of right hand and 100 cc. of left hand together received 18.88 grams of blood per minute before the ligation and 18.82 grams after ligation, exactly the same amount. But the distribution is totally different. Of course, this extremely exact correspondence is accidental, but it cannot be accidental that the flow in the left hand should have been so much smaller than that in the right before the operation and should have been so greatly increased after it. The cutting off of the path through the innominate and right common carotid obviously permitted more blood to enter the alternative route of the left subclavian and left carotid. That the flow in the left carotid was increased after the operation was indicated by the plainly visible throbbing of the left temporal artery. I have elsewhere discussed[3] the reciprocal effect of occlusion of one path upon the corresponding vascular path on the other side of the body.

The next blood flow examination was made on May 28 (seventeen days after the ligation). The flow in the right hand was 4.76 grams, and in the left 15.31 grams per 100 cc. per minute (for a period of five minutes when the flows were at the maximum for the two hands), with room temperature 26.0° C. The ratio of the

[3] Stewart, *Jour. Exper. Med.*, 1915, xxii, 1.

flows was 1 to 3.21, indicating a steady improvement in the collateral circulation. Including a period of vasoconstriction due to a psychical cause, which of course diminished the circulation more in the left hand than in the right, the flows (for ten minutes) were 4.15 grams per 100 cc. per minute for the right and 12.17 grams for the left hand (ratio 1 to 2.93). The reflex change in the flow elicited in the right hand by immersing the left hand in warm water was small, as is always the case in a part whose circulation is mechanically obstructed. For the three minutes immediately preceding the vasomotor test the flow in the right hand was 4.04 grams per 100 cc. per minute. For the first four minutes of immersion of the left hand in warm water the flow in the right sank to 3.05 grams per 100 cc. per minute, to rise to 4.86 grams per 100 cc. per minute for the remaining four minutes of the period, an insignificant reaction.

On June 4 (twenty-four days after the operation) the flow in the right hand was 4.86 grams and in the left 9.00 grams (ratio 1 to 1.85) per 100 cc. per minute for the last 18 minutes in the calorimeters, with room temperature 23.9° C. The patient came to the hospital for the examination on rather a cool morning, naturally with bare hands, and vasoconstriction due to this was probably responsible for cutting down the flow in the left hand. For the reason already given the effect on the right hand would be comparatively insignificant. The ratio is therefore probably to some extent artificial, and gives an unduly favorable view of the development of the collateral circulation at this time. Nevertheless the fact that in spite of the vasoconstriction the flow in the right hand is absolutely greater than at the last examination shows clearly enough that the collateral circulation is still opening up.

The last examination was made on June 11 (thirty-one days after the operation). The right hand was now being freely used, the only symptoms which troubled the patient being numbness along the palmar surface of the thumb and the radial surface of the index finger. The hand was fairly strong, although not of course as strong as the left hand. The flow in the right hand was 8.55 grams per 100 cc. per minute and in the left 14.24 grams (ratio 1 to 1.66). There was no pulse in the accessible arteries of the right anterior extremity.

The collateral circulation has therefore developed much more rapidly than in the other case. This is doubtless to be attributed in part at least to the youth of the patient and the consequent greater distensibility of his arteries and the greater driving power of his heart.

Protocols.

First Examination of Blood Flow.—Arthur B. May 8, 1915. Hands in bath at 10.27 a. m., in calorimeters at 10.38½, out of calorimeters at 10.51. Pulse 84.

Time.	Temperature of			Time.	Temperature of		
	Calorimeters.		Room.		Calorimeters.		Room.
	Right.	Left.			Right.	Left.	
10.38	31.31	31.21		10.46	31.73	31.39	
10.40	31.36	31.22		10.47	31.79	31.41	22.1
10.41	31.38	31.25	21.4	10.48	31.84	31.425	
10.42	31.42	31.26	21.9	10.49	31.92	31.45	22.5
10.43	31.49	31.28		10.50	31.99	31.47	
10.44	31.56	31.30		10.51	32.05	31.495	22.9
10.45	31.64	31.33		11.02	31.89	31.33	

Cooling of calorimeters in 11 minutes, right 0.16°, left 0.165°. Volume of right hand 482 cc., of left 427 cc. Water equivalent of calorimeters with contents, right 3,480, left 3,436. Rectal temperature 37.65° C.

Second Examination.—May 22, 1915. Hands in bath at 2.11 p. m., in calorimeters at 2.20, out of calorimeters at 2.31. Pulse 100.

Time.	Temperature of			Time.	Temperature of		
	Calorimeters.		Room.		Calorimeters.		Room.
	Right.	Left.			Right.	Left.	
2.19	31.98	31.98		2.27	32.015	32.41	
2.21	31.96	32.02		2.28	32.03	32.48	25.1
2.22	31.97	32.08	25.0	2.29	32.04	32.55	
2.23	31.97	32.14		2.30	32.055	32.62	25.0
2.24	31.975	32.20	25.0	2.31	32.07	32.68	
2.25	31.98	32.26		2.43	31.95	32.54	
2.26	32.00	32.34	25.0				

Cooling of calorimeters in 12 minutes, right 0.12°, left 0.14°. Volume of right hand 485 cc., of left 410 cc. The mark on the left wrist was inadvertently put somewhat lower than usual, so that a somewhat smaller volume of the left hand was in the calorimeter. Water equivalent of calorimeters with contents, right 3,483, left 3,423 cc. Mouth temperature 37.0° C.

Third Examination.—May 28, 1915. Hands in bath at 2.10½ p. m., in calorimeters at 2.22. At 2.36 the left hand was immersed in water at 44° C. At 2.44 the right hand was removed from the calorimeter.

Time.	Temperature of			Time.	Temperature of		
	Calorimeters.		Room.		Calorimeters.		Room.
	Right.	Left.			Right.	Left.	
2.21	31.95	31.95		2.34	32.09	32.58	25.9
2.23	31.945	31.99	25.9	2.35	32.105	32.63	
2.24	31.96	32.04		2.36	32.12	32.67	
2.25	31.97	32.08	26.0	2.37	32.125		25.7
2.26	31.975	32.12		2.38	32.135		
2.27	31.985	32.16	26.0	2.39	32.14		
2.28	31.99	32.21		2.40	32.15		25.9
2.29	32.005	32.27	26.1	2.41	32.165		
2.30	32.02	32.34		2.42	32.185		
2.31	32.04	32.40	26.1	2.43	32.205		25.9
2.32	32.055	32.47		2.44	32.22		
2.33*	32.08	32.54		2.52	32.14	32.48	

* Here he began to concern himself about the preparations being made for the warm water test, causing some psychical vasoconstriction.

Cooling of calorimeters, right 0.08° in 8 minutes, left 0.19° in 16 minutes. Volume of right hand 458 cc., of left 420 cc. Water equivalent of calorimeters with contents, right 3,461, left 3,431 cc. Rectal temperature 37.44° C.

Fourth Examination.—June 4, 1915. The day was rather cool and the examination was begun soon after his arrival at the hospital. Pulse 78. Hands in bath at 11.33 a. m., in calorimeters at 11.42½, out of calorimeters at 12.05.

Time.	Temperature of			Time.	Temperature of		
	Calorimeters.		Room.		Calorimeters.		Room.
	Right.	Left.			Right.	Left.	
11.42	31.70	31.65		11.56	31.805	31.975	
11.44	31.68	31.66	23.6	11.57	31.82	32.01	
11.45	31.69	31.67	23.8	11.58	31.83	32.04	23.9
11.46	31.695	31.675	23.8	11.59	31.845	32.07	
11.47	31.70	31.70		12.00	31.855	32.09	
11.48	31.71	31.74	24.0	12.01*	31.875	32.14	24.0
11.49	31.73	31.78		12.02	31.895	32.175	
11.50	31.74	31.80	23.9	12.03	31.91	32.19	24.0
11.51	31.75	31.83	24.0	12.04	31.925	32.23	
11.52	31.755	31.85		12.05	31.95	32.275	
11.53	31.76	31.88		12.13	31.84	32.16	
11.54	31.775	31.92	23.9				
11.55	31.795	31.95					

*Here he is beginning to fidget and says the right arm and hand are getting tired.

Cooling of calorimeters in 8 minutes, right 0.11°, left 0.115°. Volume of right hand 446 cc., of left 413 cc. Water equivalent of calorimeters with contents, right 3,452, left 3,425. Rectal temperature 37.19° C.

Fifth Examination.—June 11, 1915. Pulse 84. Hands in bath at 11.03 a. m., in calorimeters at 11.12¾, out of calorimeters at 11.30.

Time.	Temperature of			Time.	Temperature of		
	Calorimeters.		Room.		Calorimeters.		Room.
	Right.	Left.			Right.	Left.	
11.12	31.77	31.74		11.23	32.10	32.29	
11.14	31.80	31.79	25.0	11.24	32.13	32.35	25.1
11.15	31.84	31.85	25.1	11.25	32.165	32.41	
11.16	31.88	31.91		11.26	32.20	32.46	25.1
11.17	31.905	31.97	25.0	11.27	32.24	32.515	
11.18	31.93	32.02		11.28	32.26	32.56	
11.19	31.965	32.075		11.29	32.29	32.61	
11.20	32.00	32.135	25.0	11.30	32.32	32.66	
11.21	32.04	32.18		11.37	32.235	32.57	
11.22	32.07	32.24					

Cooling of calorimeters in 7 minutes, right 0.085°, left 0.09°. Volume of right hand 457 cc., of left 425 cc. Water equivalent of calorimeters with contents, right 3,460, left 3,435 cc. Rectal temperature 36.91° C.

SUMMARY.

The development of the collateral circulation after ligation of the innominate and right common carotid arteries for subclavian aneurysm was studied in two cases by measuring the rate of blood flow in the hands from time to time.

In a woman, sixty-eight years old, the flow in the right hand three weeks after the operation was two-sevenths of that in the left. Nineteen weeks after the operation the flow in the right hand was more than three-fourths of that in the left, although no pulse returned until long afterwards.

In a man, twenty-five years old, the flow in the right hand eleven days after the operation was between one-fourth and one-fifth of that in the left. Seventeen days after the operation the flow in the right hand was nearly one-third of the flow in the left. Twenty-four days after the operation the flow in the right hand had increased to more than one-half of the left hand flow. Thirty-one days after the operation the flow in the right hand was three-fifths of that in the left, without return, as yet, of any pulsation.

Before the operation the flow in the right hand was markedly greater than in the left, notwithstanding the small size of the right radial pulse as compared with the left. The explanation of this fact is discussed.

THE ACCELERATION OF ESTERASE ACTION.

Studies on Ferment Action. XXVIII.

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The importance ascribed by various authors to the ferments of the body in the processes of immunity makes it important to study not only the ferments normally present and their activity under normal conditions, but also to find means by which they can be increased in amount, or, failing in this, to bring about a greater activity of those present.

We have already shown (1) that the antiferments of the serum, of the cells of the body, and of bacterial cells, are composed of unsaturated fatty acids probably in the form of esters, and that oxidation of these acids renders them inactive. It is probable that dissociation of the esters might also render them inactive as antiferments, as the activity of the acids is dependent upon their degree of dispersion, and this probably would be lessened by dissociation owing to their relative insolubility. Bearing this in mind we should naturally attach considerable importance to any ferment, such as esterase, which can cause a dissociation of esters; for the removal of the protective esters from bacteria may render them more susceptible to the action of proteolytic ferments, which in turn, by hydrolysis, can render the toxic substances non-toxic. It is for this reason that we wish to report upon a group of substances, which without bearing a very definite relation to one another, have the ability to accelerate greatly the action of both serum and tissue esterases. A great deal of work has been devoted to the study of substances which accelerate the action of lipase and esterase, particularly by Biondi (2), Magnus (3), Loevenhart (4), von Hess (5),

Falk (6), Whipple (7), and Quinan (8), but so far as we are aware, none of these authors has worked with the agents which we have found to be the most active.

Technique.

The tissue esterase was prepared from dogs' livers. The livers were cut up in a meat machine and then extracted with glycerine for four days in the thermostat. The resulting mass was filtered first through cotton and then through filter paper, and the filtrate saturated with ammonium sulphate. The precipitate thus obtained was redissolved in water and reprecipitated several times with ammonium sulphate until the filtrate became colorless. The final precipitate was dissolved in a small amount of water and dialyzed against running water until it failed to react with either barium hydrate or Nessler's solution. The preparation was again filtered through filter paper, a small amount of toluol was added, and it was then stored in the ice box. 1 cc. of the esterase prepared in this manner when incubated for four hours with 1 cc. of ethyl butyrate usually gave an acidity equal to 7 cc. of N/100 sodium hydrate. We attempted to concentrate further the esterase by fractional precipitation with ammonium sulphate, but were not very successful. Esterases prepared in this manner and kept in the ice box were just as active after three months as they were originally. Serum esterase from dogs, rabbits, and guinea pigs was also used.

The experiments were conducted in 150 cc. Erlenmeyer flasks. The agent to be tested was put into the flask with the esterase, the volume brought up to 10 cc. with 0.9 per cent sodium chloride solution, and 1 cc. of neutral ethyl butyrate added. Various control flasks containing the agent alone, the agent plus esterase but without ethyl butyrate, and the agent plus ethyl butyrate but without esterase were set up at the same time. The flasks after the addition of 0.5 cc. of toluol to each were tightly stoppered, shaken 100 times, and placed in the incubator for four hours. 25 cc. of neutral 95 per cent ethyl alcohol were then added to each flask, and the acidity was determined by titrating against N/50 sodium hydrate, with phenolphthalein as the indicator. In the tables, however, the results are expressed in the number of cc. of N/100 sodium hydrate required to

neutralize the mixtures after four hours' incubation. These figures show the increase in acidity obtained after deducting that found in the control flasks, and in each instance represent the average of several experiments.

It was noted that esterases prepared from different livers were not always activated to the same degree by the various reagents used. In several instances esterases which were considered weak preparations when acting alone gave much higher figures in the presence of certain reagents than ones which were considered to be much stronger. Thus one preparation which gave an acidity equal to 8 cc. N/100 sodium hydrate when incubated for four hours with 1 cc. of ethyl butyrate gave 70 cc. in the presence of sodium citrate, while another preparation which gave 7 cc. of acidity with ethyl butyrate alone, gave only 40 cc. when incubated with sodium citrate solution.

EXPERIMENTS.

Many substances have been tested during the progress of the work, but we shall discuss only those which exert a definite accelerating action.

The most active agent with which we have worked is sodium citrate. As far as we are aware, there are no statements in the literature concerning the accelerating action this salt exerts on the activity of esterase. Falk (6) discovered that sodium acetate accelerated the action of vegetable lipase, and Quinan (8) observed that it also accelerated the action of tissue esterases. Quinan found that the specific action is a progressive one which increases with increasing concentrations of the agent. Table I indicates that the accelerating of sodium citrate also increases with increasing concentrations of the salt, and that it is far more active than any accelerating agent yet reported.

Table I shows the accelerating action of different amounts of sodium citrate on liver esterase. The figures represent the excess of acidity over that obtained with esterase alone.

As shown in Table I, sodium citrate both in large and small amounts markedly increases esterase action. Potassium citrate is also very active as an accelerating agent, but the sodium salt is more so. As both potassium and sodium citrate accelerate the action of

the ferment, experiments were made to determine if the citric acid was the activating part of the salt, but it was found to have little if any stimulating action.

TABLE I.

Amount used. *gm.*	Increased acidity. *cc. N/100 NaOH*
1.0	110
0.8	106
0.6	103
0.4	98
0.2	90
0.1	74
0.01	18
0.001	2
0.0001	0

The fact that sodium and potassium citrate inhibit the coagulation of blood led us to test other substances which influence coagulation. Of those which prevent coagulation we used potassium oxalate, hirudin, and cobra venom, while calcium lactate represented those which accelerate coagulation. Of course, we are aware that these substances probably do not act in the same manner in preventing coagulation.

Cobra venom and hirudin were found to be slightly active in accelerating esterase action, while calcium lactate and potassium oxalate were very active. It will be seen that these reagents accelerate esterase action regardless of their influence on coagulation. Table II shows the activating action of calcium lactate and potassium oxalate. The figures show the increase in acidity above that obtained with the esterase alone, which was 6.7 cc.

TABLE II.

Action of Calcium Lactate and Potassium Oxalate on Esterase Action.

Amount used. *gm.*	Potassium oxalate. *cc. N/100 NaOH*	Calcium lactate. *cc. N/100 NaOH*
1.0	40.5	33
0.8	55.0	35
0.6	52.5	38
0.4	40.5	35
0.2	28.5	20
0.1	22.5	10
0.01	6.8	6
0.001	1.0	1
0.0001	0	0

In view of the activating action of sodium citrate *in vitro,* we made some experiments to see if it would act in a similar manner *in vivo.*

Dog 1.—Weight 5 kilos. Was given 2 gm. of sodium citrate intravenously. Animal immediately developed dyspnea, the pupils became dilated, and it was evidently in distress. Complete recovery within 1 hour.

Table III shows the activity of the serum before and after injection. The figures show the acidity remaining after subtracting the controls.

TABLE III.

Amount of serum. *cc.*	Time.	Results. *cc. N/100 NaOH*
1	Before injection	0.4
1	15 min. after injection	0.8
1	1 hour " "	0.1
1	5 hours " "	0.8

The changes are not marked. As the citrate is very toxic when given intravenously, another experiment was conducted in which 5 gm. were injected into the peritoneal cavity of a dog.

Dog 2.—Weight 5 kilos. Injected with 5 gm. of sodium citrate dissolved in sterile salt solution into the peritoneal cavity. After the injection the dog immediately became very sick and restless, the abdomen became distended, and apparently there was considerable pain. The animal was killed two hours after the injection, as it was almost dead. Blood was obtained from the hepatic vein and from the heart. Considerable peritoneal exudate was present, and this was also tested. Table IV shows the activity of the serum esterase before and after the injection of sodium citrate. The controls have been subtracted.

TABLE IV.

Amount of serum. *cc.*	Time.	Results *cc. N/100 NaOH*
1	Before injection	0.8
1	(Heart) 2 hours after injection (dead)	1.0
1	(Liver) " " " " "	1.4
1	Peritoneal exudate	0.4

In both instances toxic symptoms were observed and a definite though slight rise in serum esterase followed. This rise may have been due either to the presence of the citrate in the serum, or to mobilization of the ferment as a result of the injury to the cells of the body.

CONCLUSIONS.

1. Potassium citrate, potassium oxalate, and calcium lactate accelerate the action of tissue and serum esterases.

2. Intravenous and intraperitoneal injections of large amounts of sodium citrate do not cause a definite increase in the activity of the serum esterase.

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THE RELATION OF SERUM ESTERASE TO LIVER DESTRUCTION.

Studies on Ferment Action. XXIX.

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The variations of esterase in the blood of animals which have been poisoned by phosphorus or chloroform have been studied by Whipple (1), von Hess (2), and Loevenhart (3). Whipple observed that the esterase is increased in strength in destructive conditions of the liver, the degree of increase bearing a definite relation to the extensiveness of the process. He concludes that the increase is due to the liberation of the ferment from the liver cells which are undergoing disintegration. In certain of his experiments he tied the larger vessels of the liver, but still observed the increase in the general circulation. He explains these results by assuming the presence of collateral vessels which carry the ferment to the general circulation. Just about the time our experiments were completed an article appeared by Quinan (4) in which he reports the results of his study of tissues of healthy dogs and dogs poisoned with phosphorus and chloroform. He found that in the poisoned animals the esterase content of the liver cells was decreased 27 to 38 per cent, while kidney and muscle tissue showed a corresponding increase. He concludes that the tissues containing the largest amount of esterase must be the ones which furnish the excess ferment in the serum in chloroform and phosphorus poisoning.

Technique.

The work was conducted with blood obtained from various sources from animals poisoned with chloroform and phosphorus. In each instance blood was drawn from the ear of the dog about one hour before the chloroform or phosphorus was administered. This blood was assumed to represent the normal esterase strength of that particular animal. In some animals other tests were made on the same day, but more frequently a test was made each subsequent day until the animal died or was killed. The animals were anesthetized on the day the serum esterase reached its maximum strength, and

blood was drawn from the portal and hepatic veins, the inferior vena cava below and above the entrance of the hepatic vein, and from the heart. This procedure was adopted in order to get the blood from the liver when presumably it would contain the most esterase. At this time the hepatic blood should contain an amount much larger than that present in the general circulation owing to its subsequent dilution.

The chloroform was given subcutaneously in olive oil to dogs in the proportion of 2 cc. of chloroform for each 1,000 gm. The phosphorus oil was given in 2 cc. doses on two successive days. The degree of fatty degeneration of the livers varied; in some it was very marked, in others slight.

The livers were ground up in a meat machine, weighed, and extracted with glycerine for seventy-two hours in the incubator. The mass was then diluted with two volumes of water and filtered until clear. The clear filtrate was saturated with ammonium sulphate, and the precipitate redissolved and reprecipitated several times. It was finally dissolved in a small amount of water, dialyzed free from ammonium sulphate, and its esterase activity determined. The esterase activity of the solutions thus prepared is represented as the activity of 1 gram of the original tissue. No doubt some of the ferment is lost by these procedures, but the loss is probably about the same in all. In any case the differences are so striking that our interpretation can hardly be wrong. For purposes of comparison extracts from normal livers were prepared in the same manner. The technique of determining the esterase strength has been described in a previous paper (5).

Esterase Content of Normal and Injured Liver Tissue.

Dog 1.—Normal. Killed, the liver removed, and treated as already described. The esterase activity of 1 gm. of liver tissue gave an acidity equal to 104.4 cc. of N/100 NaOH when incubated for 4 hours with neutral ethyl butyrate. A number of normal livers have been examined in the same manner, and while the results were not the same in all cases, the esterase activity per gm. of weight was always greatly in excess of that obtained with the livers of poisoned animals.

Dog 2.—Received 3 cc. of phosphorus oil on Apr. 3. Animal anesthetized Apr. 6. The liver showed marked fatty degeneration. The esterase activity per gm. of weight of the liver was equal to 53.4 cc. N/100 NaOH.

Dog 3.—Received 10 cc. of chloroform in olive oil subcutaneously. Animal anesthetized the following day. The liver showed marked fatty degeneration. The esterase activity per gm. of weight was equal to 39 cc. N/100 NaOH.

As will be seen from the above experiments, which are merely examples of others, the esterase activity of normal liver tissue gave an acidity equal to 104.4 cc. N/100 NaOH, the phosphorus liver 53.4 cc., and the chloroform liver 39 cc. In other words, the esterase activity of normal liver tissue is two to three times greater than that from liver tissue obtained from animals poisoned with phosphorus or chloroform. This confirms the observations of Quinan.

Changes in Serum Esterase.

Dog 29.—Weight 6 kilos. Inoculated subcutaneously daily for three days beginning Mar. 18, 1915, with 1 cc. of oil of phosphorus. It was anesthetized the day following the last dose, and blood drawn from the portal vein, the hepatic vein, the inferior vena cava, and from the heart. Table I gives the esterase activity of the specimens of blood.

TABLE I.

Source of blood.	Esterase activity. *cc. N/100 NaOH*
Heart	3.7
Portal vein	4.8
Inferior vena cava	4.7
Hepatic vein	3.3

In this dog the destruction of liver tissue was not very marked, and there was but little increase in the activity of the serum esterase. However, even here, with a slight degree of liver destruction, the esterase action of the blood from the hepatic vein was less than that obtained from other sources. The blood from the portal vein and inferior vena cava was most active.

Dog 49.—Weight 7.3 kilos. Inoculated daily for three days beginning Mar. 29, 1915, with 1 cc. of phosphorus oil. It was anesthetized the day after the last dose was given, and blood was obtained from the portal vein, hepatic vein, inferior vena cava, and the heart. The liver showed a mild degree of fatty degeneration. Table II gives the esterase activity of the blood.

TABLE II.

Source of blood.	Esterase activity. *cc. N/100 NaOH*
Heart	2.4
Portal vein	Lost.
Inferior vena cava	2.6
Hepatic vein	2.0

Here, also, the esterase activity of the blood obtained from the hepatic vein is less than that present in the blood from other sources.

Dog 59.—Weight 6.3 kilos. Inoculated subcutaneously daily for three days beginning Apr. 3, 1915, with 1 cc. of phosphorus oil. The animal was anesthetized the day following the last dose. The liver showed marked fatty degeneration. Table III gives the esterase activity of the different specimens of blood.

TABLE III.

Source of blood.	Esterase activity. *cc. N/100 NaOH*
Heart	19.0
Portal vein	21.0
Inferior vena cava, below liver	16.5
" " " above "	15.5
Hepatic vein	14.0

This animal, with extensive liver changes, shows a very high esterase action of the serum. This is particularly evident in the portal blood, while the least active is the blood from the hepatic vein. This decrease in the activity of the blood from the hepatic vein is interesting as it is less than either the portal or arterial blood, and suggests the possibility of the esterase being adsorbed or destroyed as it passes through the liver.

Dog 60.—Weight 6.2 kilos. Inoculated subcutaneously on Apr. 5, 1915, with 2 cc. of phosphorus oil, and on Apr. 6 with 1 cc. The animal was anesthetized on the day following the last dose. The liver presented a high degree of fatty degeneration. Table IV gives the esterase activity of the blood from different sources.

TABLE IV.

Source of blood.	Esterase activity. *cc. N/100 NaOH*
Heart	7.3
Portal vein	7.3
Inferior vena cava	8.1
Hepatic vein	6.1

Here, also, the blood from the hepatic vein was less active. In this case the blood from the inferior vena cava was more active than that from the portal vein.

Dog 64.—Weight 3.5 kilos. Inoculated subcutaneously Apr. 15, 1915 at 9 a. m., with 10 cc. of chloroform in olive oil. The animal was anesthetized the following day. The serum of the animal obtained before the inoculation gave an acidity with ethyl butyrate of 0.4 cc. N/100 NaOH after 4 hours' incubation,

while that obtained at 4 p. m. of the same day gave an acidity equal to 12.4 cc. Table V gives the esterase activity of the blood.

TABLE V.

Source of blood.	Esterase activity. *cc. N/100 NaOH*
Heart	18.6
Portal vein	16.3
Inferior vena cava, below liver	19.7
" " " above "	17.3
Hepatic vein	16.3

This animal had extensive fatty degeneration of the liver and the serum had a high esterase content. There was a general rise in esterase action until the animal was killed. In seven hours it increased from 0.3 to 12.4 cc., with a further increase of 6.6 cc., during the next sixteen hours. Here again the esterase activity of the blood from the hepatic vein is low, but it is just as strong as that from the portal vein. The blood from the inferior vena cava is the most active.

DISCUSSION.

Quinan's results (4) show that in chloroform and phosphorus poisoning there is a decrease in the esterase strength of the liver cells, while it is increased in the kidney and muscle tissue. He does not prove, however, that the increase in serum esterase may not be due to a mobilization of the ferment from the disintegrating liver cells. The decrease of ferment in the liver might be due to its rapid mobilization.

It is evident, however, from the results of the experiments just described, that the increase in serum esterase in animals poisoned with chloroform or phosphorus cannot be due to a mobilization of the ferment owing to the destruction of the liver cells. If this were true we should expect to find the esterase strength of the blood from the hepatic vein higher than that from other portions of the body, but the reverse holds true. In fact, it would seem that the injured liver cells actually adsorb or destroy some of the excess esterase, as the blood from the hepatic vein contains less than is found elsewhere. The fact that the excess serum esterase in these conditions is not derived from the degenerating liver cells affords a rational explanation of the results obtained by Whipple (1) in Eck fistula dogs,

where the hepatic artery, with all its branches, has been ligated. This author found that the serum esterases were increased in all conditions accompanied with destruction of liver cells. If this holds true, we must assume either that the agent causing the destruction of the liver cells causes also a mobilization of the ferment from other sources, or that the products of the disintegration of the liver cells enter the general circulation and bring about this mobilization. This view is confirmed by the results of some of the experiments reported in our work on the effects of the intravenous injection of protein cleavage products, where we found that the serum esterase activity was greatly increased. In Whipple's experiments it is probable that the products of cell disintegration are removed by the lymphatics, and in this way get into the general circulation. Quinan has shown that in these conditions the esterases are increased in the kidneys and muscles, and so it is possible that they are derived from these tissues, but in several of our experiments we observed the highest esterase action in the portal blood, so this source must also be considered.

CONCLUSIONS.

1. Liver tissue showing fatty degeneration obtained from animals poisoned with phosphorus or chloroform contains a decreased amount of esterase.

2. The serum of animals poisoned with phosphorus or chloroform has a high esterase activity.

3. The increased amount of esterase in the serum is not derived from the disintegrating liver cells as the esterase in the blood of the hepatic vein is less than that found elsewhere.

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FURTHER INVESTIGATIONS ON THE ORIGIN OF TUMORS IN MICE.

II. Tumor Incidence and Tumor Age in Hybrids.

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We wish to report the results that we obtained in crossing the strains described in our preceding paper.[1] We used some of the hybrids thus obtained for further hybridization. Soon after the beginning of our studies of heredity in cancer in mice, we started experiments in hybridization in order to determine whether tendency to a high or a low tumor rate prevails in the offspring of two parents, one of which had a high, while the other had a low tumor rate; or, to state it differently, whether a tendency to develop cancer is a dominant or recessive character.[2] We referred to these experiments briefly in a former publication.[3] We stated that in mating a certain strain rich in tumors with a strain very poor in tumors we obtained hybrids which have so far been rich in tumors. In this case the male, which is itself not liable to have tumors, transmits the liability to have tumors to the daughters. We mentioned another strain rich in tumors which if mated to a strain poor in tumors produced hybrids rich in tumors. We stated that we intended to collect further data before arriving at a definite conclusion. We can now on the basis of a large number of experiments confirm our previous preliminary conclusion that the higher tumor rate may be dominant in crosses. But we found that the result of hybridization varies in

[1] Lathrop, A. E. C., and Loeb, L., *Jour. Exper. Med.*, 1915, xxii, 646.

[2] We use the terms "dominant," "recessive," and "unit factor" at present merely as convenient means of expression, without implying by the use of these terms the adoption of further going hypotheses concerning the mechanism of heredity in cancer.

[3] Lathrop, A. E. C., and Loeb, L., *Proc. Soc. Exper. Biol. and Med.*, 1913, xi, 34.

different crosses. We again gave special attention to the inheritance of the tumor age. Yet while the material on which our further conclusions are based is large, we repeat what we said in the preceding paper, that we are still continuing our experiments and that we are ready to modify our conclusions whenever our further work should fail to confirm our first results. We lay special stress on communicating the observations as such and regard some of our conclusions as only tentative. We take this attitude especially as we were often obliged, through lack of sufficient funds in this work, to direct the breeding in such a way that simultaneously with our scientific aims economic needs were satisfied. Otherwise the experiments could not have been completed.

Hybrids Produced.

European + 102 and 103: 16% (16%) + 30% (38%). I age class + II age class.[4]

Hybrids: total 21% (25%). Mixture of 102 and 103: 22% (26%), 29% (32%), 29% (33%). European + 102: 14% (18%), 17% (21%). European + 103: 10%. IV age class.

The European mice were imported. 102 and 103 were the offspring of an old male, the son of a tumor mouse, and plum-silver females which had mixed origin and gave origin to the No. 8 strain. The old male was later mated to his daughters (among them 102 and 103) and their offspring constituted the 8½ strain. 102 and 103 after having been mated to the European had young ones at the same time and their offspring could only be partly distinguished as to whether 102 or 103 was the mother of a certain mouse. 102 and 103 are there-

[4] Before discussing in each case the result of the hybridization, we mention the two parent strains which entered into the cross; the male parent is named first, then the female parent. In the case of the first group mentioned the European was the male and 102 or 103 the female parent. Then follows the tumor rate of the father, 16% (including all age classes), and the tumor rate of the II and III age classes only, in brackets (16%) of the father strain; in the case of the mother strain the figures are 30% for all age classes and 38% for the II and III age class. Then follows the age class of the father strain: I age class; and of the mother strain: II age class. In giving the tumor rate of the hybrids the figure for the second and third age class follows again in brackets. The age class of the hybrids is given last. In the case of the first hybrid group several figures are given for the tumor rate of the hybrids; in some of the offspring 102, in others 103 was the mother, in still others the offspring of 102 and 103 were mixed. The lists of the mice with and without tumors in the different generations and in different age classes are arranged in the same way as in the preceding paper (Lathrop and Loeb, *Jour. Exper. Med., loc. cit.*). On the left side of the page are the mice without, on the right side the mice with tumors; the Roman figures signify the age classes.

fore essentially of a similar character to the No. 8 and No. 8½ strain. Altogether a record was kept of 146 mice of this strain. The variations among the different strains and different families are relatively small. The tumor rate throughout is low, on the whole similar to European, or intermediate between the European and No. 8. The tumors appear late, and belong to the IV age class. They herein differ from their parents, where the tumors appeared early; namely, in the I and II age class. Both parents of these hybrids had a low or medium tumor rate, and the tumor rate of the hybrids agrees with that of the parents. In several cases these hybrids were used for further hybridizations.

(a) (European + 102) F_1 + 8½ F_4: 14% (18%), 17% (21%) + 17% (19%). IV + II age class.

In this case both parents had a similar, low tumor rate; the tumor rate of the hybrids is the same as that of the parents, 16% (18%). The tumors appear late, the fourth is dominant over the second age class. Five generations of these hybrids were observed, and the results in all agreed with each other in regard to tumor rate as well as to tumor age.

	Without tumors.	With tumors.
F_1	26 (3 I 8 II 15 III)	5 (2 I 3 III)
	84% (12% I 31% II 57% III)	16% (40% I 60% III)
F_2	96 (18 I 26 II 52 III)	26 (2 I 7 II 17 III)
	79% (17% I 27% II 56% III)	21% (8% I 27% II 65% III)
F_3	210 (49 I 66 II 95 III)	40 (4 I 11 II 25 III)
	84% (23% I 31% II 46% III)	16% (10% I 27% II 63% III)
F_4	47 (19 I 9 II 19 III)	3 (3 III)
	94% (40½% I 19% II 40½% III)	6% (100% III)
F_5	16 (11 I 3 II 2 III)	3 (1 II 2 III)
	84% (70% I 18% II 12% III)	16% (34% II 66% III)
Total:	396 (101 I 112 II 183 III)	77 (8 I 19 II 50 III)
	84% (25% I 28% II 47% III)	16% (10% I 25% II 65% III)
II + III	82%	II + III 18%

(b) 101 (English) + (European + 103): 71% (72%) + 10% or total 21% (25%). I age class + IV age class.

In this case males from a strain rich in tumors (101 English) were mated to females from a strain poor in tumors (European + 103). In the strain of the father the tumors appeared early, in the strain of the mother late. In the hybrids the tumor rate is intermediate, 34% (46%). The tumors in the offspring belong to the IV age class; the late appearance of the tumors seems again to be dominant. The hybrid mice were observed through three generations and the results in the various generations agreed well, on the whole. The figures for the sum of all the generations are as follows:

Without tumors.	With tumors.
101 (49 I 25 II 27 III)	51 (61 I 27 II 18 III)
66% (49% I 25% II 26% III)	34% (12% I 52% II 36% III)
II + III 54% III 60%	II + III 46% III 40%

(c) London + (European + 103) F_2: 28% (27%) + 10% (European + 103) or + 21% (25%) European + 102 and 103 (total). II age class + IV age class.

The parents have here a low or medium low tumor rate. In the father the tumors belonged to the II, in the mother to the IV age class. In the hybrids the tumor rate is low (5%), even slightly lower than in the mother. The tumors appear late.

The total figures are:

Without tumors.	With tumors.
74 (12 I 31 II 31 III)	4 (2 II 2 III)
95% (16% I 42% II 42% III)	5% (50% II 50% III)

The IV age class is again dominant.

(d) (European + 103) F_1 + III daughter of No. 10 : 10% or total 21% (25%) + ?30% (38%). IV age class + ?II age class.

In order to understand the character of these and the following hybrids, it will be necessary to state the character of the family of No. 10. No. 10 was a female mouse which was found to have a tumor on Sept. 8, 1910. She belonged probably to the No. 8 strain. She was in all probability mated to a male from the No. 8 strain. Sept. 10, 1910, she gave birth to three daughters, which are designated as follows: I (Nov. 3d), II (Nov. 8th), and III. The third daughter failed to grow for a considerable period of time, but later grew to normal size. Daughter I (Nov. 3d) was first mated to a European male 151, afterwards to an English Silver male, and last to a Cream male. Daughter II (Nov. 8th) was first mated to the same European male 151, and later to a male from the No. 8½ strain. The III daughter was mated to a son of European + 103. The European 151 was later crossed with females of No. 8 F_5. These various crosses are represented in Table I.

TABLE I.

No. 10 ♀ + No. ?8 ♂

151 European ♂ mated to:

♀ I Nov. 3d	♀ II Nov. 8th	♀ III
(a) + I Nov. 3d ♀	(a) + 151 European ♂	(a) + 151 European ♂	(a) + (European + 103) F_1 ♂
(b) + II Nov. 8th ♀	(b) + English Silver ♂	(b) + 8½ ♂	
(c) + No. 8 F_5 ♀	(c) + Cream ♂		

The offspring from the cross (European + 103) F_1 + III daughter of No. 10 gave a tumor rate of 17% (22%). The tumors belonged to the IV age class. The tumor rate and tumor age of the father were dominant in this case. About the tumor rate of the No. 10 family we have no definite knowledge, although we must assume that the I and II daughters had a very high tumor rate.

Without tumors.	With tumors.
F_1 8 (2 II 6 III)	0
F_2 94 (35 I 22 II 37 III)	11 (4 II 7 III)
90% (37% I 23% II 40% III)	10% (36% II 64% III)
F_3 38 (15 I 13 II 10 III)	17 (3 I 7 II 7 III)
69% (39% I 34% II 27% III)	31% (18% I 41% II 41% III)
Total: 140 (50 I 37 II 53 III)	28 (3 I 11 II 14 III)
83% (36% I 27% II 37% III)	17% (11% I 39% II 50% III)
II + III 78% III 79%	II + III 22% III 21%

(e) English Sable (son of Tumor Mouse 198) + [(European + 103) F_1 + III daughter of No. 10] F_2: 70% (69%) + 17% (22%). I age class + IV age class.

In this case only a few of the offspring were obtained, but the tumor rate is high, the tumors appear late, and the hybrids belong to the IV age class.

Without tumors.	With tumors.
F_1 1 (1 I)	5 (3 II 2 III)

83% tumor rate, IV age class.

Here the high tumor rate of the English Sable male was dominant over the low tumor rate of the two females of the [(European + 103) F_1 + III daughter of No. 10] F_2 to whom they were mated. The latter had a very low tumor rate. On the other hand, it is probable that the age class (IV) of the females prevailed over the age class of the English Sable (I).

Summary.—In considering these six crosses, we shall distinguish tumor rate and age class. In two hybrids we find marked divergence of the tumor rate in the two parents; namely, 101 + (European + 103), where the tumor rate was intermediate, and English Sable + [(European + 103) F_1 + III daughter of No. 10] F_2. In this case the higher tumor rate seemed to dominate. In the other four crosses the tumor rates of the parents were either very similar to each other or differed not very markedly. In two of these cases perhaps the lower tumor rate dominated; in the two other the tumor rate of the offspring was similar to that of the parents. In the case of the age classes, on the other hand, the higher age class (later tumors) dominated in all cases. Every time the presence of the combination European + 102 or 103 called forth the IV age class, independently of the behavior of the tumor rate in the hybrids. This was already noticeable in the first cross: European + 102, 103. The age class was here higher than in either of the parent strains. It appears, therefore, that the age classes are determined by unit factors different from those determining tumor rate, and that dominance of the higher tumor rate may be combined with dominance of the higher age class (lateness of tumors).

(a) 151 (European) + No. 8 F_3: 16% (16%) (?) + 30% (38%). I age class (?) + II age class.

The hybrids had a tumor rate similar to No. 8: 30% (34%). The age class is likewise that of No. 8; *viz.*, II. The tumors appear, however, slightly earlier than corresponds to Age Class II. Four generations were observed which agreed fairly well.

Without tumors.	With tumors.
Total: 88 (31 I 19 II 38 III)	37 (8 I 20 II 9 III)
70% (35% I 22% II 43% III)	30% (22% I 54% II 24% III)
II + III 66% III 87%	II + III 34% III 19%

(b) 151 (European) + I daughter of No. 10 (Nov. 3d) : 16% (16%) (?) + high tumor rate. I age class (?) + II or III age class (?).

The hybrids show a tumor rate of 72% (81%); they belong to the II age class. We may assume that daughters I and II of No. 10 had the tendency to a high tumor rate and that it was dominant over the lower tumor rate of European. However, there is not combined with the high tumor rate an early appearance of the tumors; they belong to the II age class. The figures for these hybrids were given in our preceding paper.[5]

(c) [151 (European) + I daughter of No. 10 Nov. 3d] + 101 : F_2 72% (81%) + 71% (72%). II age class + I age class.

In this class both parents have a high tumor rate, and the hybrids have also a high tumor rate (55%), although not quite as high as the parents. This slight decrease in the tumor rate of the hybrids may be accidental, due to the relatively small number of observed mice. The tumors belong to the I age class.

Without tumors.	With tumors.
12 (8 I 2 II 2 III)	15 (10 I 5 II)
45% (66% I 17% II 17% III)	55% (66⅔% I 33⅓% II)

Four generations were observed. The results in the various generations agreed.

(d) 151 (European) + II daughter of No. 10 (Nov. 8th) : 16% (16%) (?) + high tumor rate. I age class (?) + II or III age class (?).

The II daughter of No. 10 had probably a similar tendency to a high tumor rate as the I daughter. The hybrids have here again a high tumor rate, but their rate is not quite as high as that of the preceding strain of hybrids, the offspring of the same European father and the I sister. The tumor rate of the hybrids was 65% (69%), the tumors belonged to the II age class.

Without tumors.	With tumors.
F_1 3 (3 I)	0
F_2 4 (2 I 2 II)	3 (2 I 1 III)
F_3 24 (8 I 14 II 2 III)	44 (17 I 23 II 4 III)
35% (33⅓% I 58⅓% II 8⅓% III)	65% (39% I 52% II 9% III)
F_4 3 (2 I 1 III)	15 (1 I 10 II 4 III)
16% (66⅔% I 33⅓% III)	84% (7% I 67% II 26% III)
Total: 34 (15 I 16 II 3 III)	62 (20 I 33 II 9 III)
35% (44% I 47% II 9% III)	65% (32% I 54% II 14% III)
II + III 31% III 25%	II + III 69% III 75%

The same European male and two sisters produced therefore hybrids with a similar tumor rate and a similar age class.

(e) English Silver + I daughter of No. 10 (Nov. 3d) : 7% + ? high tumor rate. I age class + ?II age class.

[5] Lathrop and Loeb, *Jour. Exper. Med., loc. cit.*

After the I daughter of No. 10 had been mated to European 151, she was mated to an English Silver male with a very low tumor rate, but belonging to an early age class. The hybrids have a tumor rate of 36% (38%) which is much higher than the tumor rate of English Silver and intermediate between the tumor rate of the English Silver and the tumor rate of the descendants of this female and the European 151 male. The tumor mice belong to the II and not to the I age class, as do the tumor mice of the English Silver. The detailed figures for the various generations of these hybrids are given in the preceding paper.[6]

(f) English Sable + (English Silver + I daughter of No. 10, Nov. 3d) : 70% (69%) + 36% (38%). I age class + II age class.

Here a strain with high tumor rate is crossed with one with medium tumor rate. The descendants have a high tumor rate, 69%, similar to the strain of the father. The tumors stand probably somewhat between the I and II age class.

Without tumors.	With tumors.
5 (4 I 1 II)	11 (5 I 6 II)
31% (80% I 20% II)	69% (45% I 55% II)

The high tumor rate is here dominant.

(g) Cream + I daughter of No. 10 (Nov. 3d) : 2% (3%) + high tumor rate. IV age class + ?II or III age class.

The hybrids have a tumor rate of 36% (38%). The tumors belong to the IV age class. Tumor rate of the hybrids is similar to the tumor rate of the English Silver + I daughter of No. 10 (Nov. 3d). English Silver and Cream have a similar tumor rate, and the hybrids also have a similar tumor rate. But while the tumors of the English Silver belonged to the first age class, the tumors of the Cream belong to the IV age class and correspondingly the tumors of the English Silver hybrids belong to the II age class, and the tumors of the Cream hybrids belong to the IV age class. As far as the age of the tumors is concerned, the higher class (lateness of the tumors) is dominant; while as far as the tumor incidence is concerned, the hybrids show an intermediate condition between both parents. Neither in this case nor in the case of the English Silver hybrids with the I daughter of No. 10 does the lower tumor rate dominate. The various generations of this hybrid strain agree very well with each other.

	Without tumors.	With tumors.
F_1	13 (1 I 2 II 10 III)	5 (5 III)
	There may be a few mice of the F_2 generation included.	
	72% (8% I 15% II 77% III)	28% (100% III)
F_2	27 (2 I 17 II 8 III)	13 (3 I 7 II 3 III)
	68% (7% I 64% II 29% III)	32% (23% I 56% II 23% III)
F_3	54 (11 I 6 II 37 III)	40 (1 I 14 II 25 III)
	58% (20% I 11% II 69% III)	42% (2½% I 35½% II 62% III)
F_4	18 (4 I 8 II 6 III)	4 (2 II 2 III)
	82% (22% I 44% II 34% III)	18% (50% II 50% III)
Total:	112 (18 I 33 II 61 III)	62 (4 I 23 II 35 III)
	64% (16% I 29% II 55% III)	36% (6% I 37% II 57% III)
	II + III 62% III 64%	II + III 38% III 36%

[6] Lathrop and Loeb, *Jour. Exper. Med., loc. cit.*

(h) No. 8½ + II daughter of No. 10 (Nov. 8th) : 17% (19%) + ? high tumor rate. II age class + ?II or III age class.

The hybrids show here a high tumor rate, similar to that of the mother, possibly slightly lower: 49% (50%). The tumors belong to the III age class. Here again it seems that greater tumor incidence and lateness of the tumors are dominant. The various generations were as follows:

	Without tumors.	With tumors.
F_1	2 (1 I 1 II)	0
F_2	5 (1 II 4 III)	2 (1 II 1 III)
F_3	27 (4 I 10 II 13 III)	28 (6 I 13 II 9 III)
	49% (15% I 36% II 49% III)	51% (21% I 47% II 32% III)
F_4	8 (3 I 2 II 3 III)	10 (5 II 5 III)
	44% (37% I 26% II 37% III)	56% (50% II 50% III)
Total:	42 (8 I 14 II 20 III)	40 (6 I 19 II 15 III)
	51% (19% I 33% II 48% III)	49% (15% I 47½% II 37½% III)
	II + III 50% III 57%	II + III 50% III 43%

(i) A subgroup of this strain was formed. After the aged 8½ male of the preceding group had died, the II daughter of No. 10 (Nov. 8th) was mated with one of her sons. [8½ + II daughter of No. 10 (Nov. 8th)] + II daughter of No. 10 (Nov. 8th) : 49% (50%) + high tumor rate. III age class + ?II or III age class.

Here the tumor rate is somewhat lower than in the preceding strain; but this is mainly due to the greater mortality in earlier periods of life in mice of this strain.

	Without tumors.	With tumors.
Total:	46 (19 I 19 II 8 III)	26 (5 I 9 II 12 III)
	64% (41% I 41% II 18% III)	36% (19% I 35% II 46% III)
	II + III 56% III 40%	II + III 44% III 60%

Summary.—In all probability there was in no instance a low tumor incidence dominant, while in several cases a high tumor rate was dominant, and in others an intermediate tumor rate prevailed. It is almost certain that the I and II daughters of No. 10 had a tendency to a high tumor rate, and we may assume that the European male 151 had a tendency to a tumor rate perhaps somewhat higher than the average European. It is interesting that the two daughters of No. 10 gave with the English Silver and Cream, which had both very low tumor rates, a lower tumor rate than with European, which had apparently a somewhat higher tumor rate. The high tumor rate is again dominant in the hybrids between English Sable and English Silver + I daughter of No. 10. The high tumor

rate is also dominant or almost dominant in the crosses between 8½ + II daughter of No. 10. The dominance of the high tumor rate in this group is, however, not complete in each case. It is of interest that the crosses with the Cream and English Silver males, in which the males had in both cases very low and similar tumor rates, showed also similar tumor rates, and that these tumor rates were lower than in the hybrids between either 8½ or European with the I or II daughter of No. 10. Again the age class of tumors appears to be transmitted to hybrids as a distinct unit factor at least partly independent of the tumor rate, and the lateness of tumors may dominate, when at the same time a high tumor rate is dominant. We found in this class undoubted instances in which a low tumor incidence did not prevail.

Hybridizations in Which the Tumor Incidence of the Two Parent Strains Differed Markedly.

(a) Grandson of Tumor Mouse 121 (English Tan) + 3 Cream females: 73% + 2% (3%). I age class + IV age class.

The Cream mothers were without tumors. The hybrids had a tumor rate intermediate between those of the parent strains; *viz.*, 42% (41%). The age class of the hybrids was also intermediate; *viz.*, II.

	Without tumors.	With tumors.
F_1	1 (1 I)	5 (3 I 2 II)
F_2	27 (12 I 9 II 6 III)	17 (5 I 7 II 5 III)
	61% (45% I 33% II 22% III)	39% (29% I 42% II 29% III)
F_3	18 (2 I 11 II 5 III)	13 (6 I 7 II)
	58% (11% I 61% II 28% III)	42% (46% I 54% II)
F_4	7 (5 I 2 II)	4 (2 I 2 II)
	64% (71% I 29% II)	36% (50% I 50% II)
Total:	53 (20 I 22 II 11 III)	39 (16 I 18 II 5 III)
	58% (37% I 42% II 21% III)	42% (41% I 46% II 13% III)
II + III	59% III 69%	II + III 41% III 31%

In this case the father that does not have a tendency to tumors influences the tumor rate of the female children.

(b) In another case an English Sable male (No. 4,444) was mated to Cream females. English Sable + Cream: 70% (69%) + 2% (3%). I age class + IV age class.

In this case the hybrids, three generations of which were observed, were without tumors. Here the low tumor incidence of the Cream was apparently dominant over the high tumor incidence of the English Sable.

Without tumors.	With tumors.
25 (7 I 11 II 7 III)	0
100% (28% I 44% II 28% III)	0%

(c) No. 8½ + English Sable: 17% (19%) + 70% (69%). II age class + I age class.

The English Sable females used in this experiment were the daughters of the male No. 4,444 used in the preceding experiment, in which the English Sable was mated to Cream females. The hybrids in Experiment (c) had a tumor rate of 61% (60%); the tumors belonged to the I age class. The high tumor incidence and early tumor age of the English are here dominant.

(d) European + English Tan (daughter of Tumor Mouse 146, granddaughter of Tumor Mouse 121): 16% (16%) + 73%. I age class + I age class.

In this case the hybrids have a tumor rate of 28% (31%). The tumors belong to the II age class. The low tumor incidence of the European seems to be dominant. The tumors appear later than in the English strain. In this case a remarkable decrease in the tumor rate is found in the third generation.

	Without tumors.	With tumors.
F_1	2 (1 II 1 III)	6 (1 I 3 II 2 III)
	25% (50% II 50% III)	75% (17% I 50% II 33% III)
F_2	21 (5 I 11 II 5 III)	12 (2 I 8 II 2 III)
	64% (24% I 52% II 24% III)	36% (17% I 66% II 17% III)
F_3	30 (8 I 11 II 11 III)	3 (2 II 1 III)
	91% (26% I 37% II 37% III)	9% (66% I 34% II)
Total:	53 (13 I 23 II 17 III)	21 (3 I 13 II 5 III)
	72% (24% I 43% II 33% III)	28% (14% I 62% II 24% III)
II + III	69%	II + III 31%

(e) Michigan Wild + English 101 F_2: (?) + 71% (72%). (?) age class + I age class.

In this experiment a male gray wild mouse, which had been caught in Michigan, was mated with English 101 females. Nothing definite is known about the tumor incidence of the strain to which the gray mouse belonged; there are, however, indications that the tumor incidence of wild gray mice is much lower than that of English 101. The offspring have here the tumor incidence and tumor age of the English 101. Hybrids 58% (70%). I age class.

	Without tumors.	With tumors.
F_1	4 (1 I 3 III)	6 (1 I 5 III)
F_2	9 (7 I 2 II)	5 (1 I 2 II 2 III)
F_3	6 (4 I 2 II)	14 (7 I 7 II)
F_4	2 (2 I)	4 (4 I)
Total:	21 (14 I 4 II 3 III)	29 (13 I 9 II 7 III)
	42% (67% I 19% II 14% III)	58% (45% I 31% II 24% III)
II + III	30%	II + III 70%

It is possible that as a result of some accident a few mice of the first generation of hybrids were the offspring of hybrid males (Michigan Wild and English

101 F_2) F_1 and the English mother: (Michigan Wild + English 101 F_2) F_1 + English 101 F_2. They may, therefore, have been ¾ instead of ½ English. But inasmuch as these particular females had no offspring, all the other generations were entirely ½ English.

(f) Cream + European 2% (3%) + 16% (16%). IV age class + I age class.

A black colored Cream male was mated to a pure European female (which became later Tumor Mouse No. 428). While No. 428 had tumors she raised three young mice, all females and very wild. These three daughters all developed tumors. In this case it is improbable that the low tumor incidence of the Cream was dominant. The higher rate of the European mother which herself had a tumor was evidently dominant.

Summary.—By combining Cream and two English strains, the tumor rate and age class in one case are intermediate, in the other case the low tumor incidence of Cream is dominant. The same English strain mated to 8½ is dominant. In a similar way the I daughter of No. 10 if mated to Cream gave a tumor rate similar to the intermediate one of the hybrids (English Tan + Cream), while the cross of the I daughter of No. 10 with a European, or of the II daughter of No. 10 with 8½, gave a high tumor rate. In a cross between European and English Tan the lower rate of the European is dominant, the tumors appear also later, while in a cross between Cream and European and probably also between a wild gray mouse and 101 English, the higher tumor incidence prevailed.

Waltzer Group.

(a) Waltzer + No. 8. In the autumn of 1909 a pure waltzing mouse was obtained from the Harvard Laboratory of Experimental Psychology. Tumors among these waltzing mice were extremely rare. This waltzing mouse was mated to a female of a Cream strain, with yellow color.[7] The offspring of this pair were black and did not waltz, a fact in accordance with the recessive character of the waltzing. These F_1 females were again mated to a pure male Waltzer. These offspring F_2 (¾ Waltzer) were in part black, some were spotted, none were waltzing. The females of F_2 were again mated to a pure male Waltzer. The offspring F_3 (⅞ Waltzer) were spotted black and white; there were no yellow mice among them; they were not waltzing.

An F_3 male (⅞ Waltzer and ⅛ Cream) was mated to white females of the No. 8 strain. Their offspring, which comprised mainly black mice or black mice with very little white are the first generation of the Waltzer + No. 8 hybrids. Mice with yellow color appeared only in the II generation of the hybrids. These mice were propagated through six generations; they were sensitive and died early and the number observed is therefore not great.

[7] These Creams were not identical with the Cream strain mentioned in the preceding paper or in other crosses, but they were equally poor in tumors.

⅞ Waltzer ⅛ Cream + No. 8: 1 to 5% (?) + 30% (38%). (?) age class + II age class.

The hybrids have a tumor rate of 31% (33%). The tumors appear early. The tumor rate of No. 8 is here apparently dominant.

Without tumors.	With tumors.
22 (12 I 4 II 6 III)	10 (5 I 4 II 1 III)
69% (55% I 18% II 27% III)	31% (50% I 40% II 10% III)
II + III 67%	II + III 33%

(b) (Waltzer + 8) + English 101 F_2: 31% (33%) + 71% (72%). Early tumor + I age class.

A male of the preceding group was mated to a female of the English 101 strain. Only a small number of the offspring were observed, but the indications are that there is a high tumor rate, probably a higher rate than in the Waltzer + No. 8.

Without tumors.	With tumors.
5 (2 I 3 II)	4 (1 II 3 III)

German Group.

In a number of crosses the German strain was used as one of the parent strains. Owing to the prevailing infection, the number of German mice observed was not very large, but the indications are that the German strain is fairly rich in tumors and that the tumors belong to the II age class.

(a) No. 8 + German: 30% (38%) + 50% (60%). II age class + II age class.

The hybrids show a tumor rate of 41% (41%). The tumors belong to the II age class. The tumor rate is therefore perhaps somewhat higher than that of No. 8 and not quite as high as that of the German mice. But the difference between the rates of the two parent strains is not great enough to make these results decisive. The age class is the same as that of the parents. The tumor rate in the second generation was in this case lower than that of the following generations.

Without tumors.	With tumors.
F_2 31 (2 I 14 II 15 III)	6 (4 II 2 III)
84% (6% I 45% II 49% III)	16% (66% II 34% III)
F_3 83 (27 I 24 II 32 III)	64 (12 I 32 II 20 III)
56% (32% I 29% II 39% III)	44% (19% I 50% II 31% III)
F_4 19 (5 I 5 II 9 III)	20 (4 I 10 II 6 III)
49% (26% I 26% II 48% III)	51% (20% I 50% II 30% III)
F_5–F_7 11 (6 I 3 II 2 III)	10 (5 I 5 II)
52% (55% I 27% II 18% III)	48% (50% I 50% II)
Total: 144 (40 I 46 II 58 III)	100 (21 I 51 II 28 III)
59% (28% I 32% II 40% III)	41% (21% I 51% II 28% III)
II + III 59% III 68%	II + III 41% III 32%

(b) German + Carter: 50% (60%) + 39% (35%). II age class + I age class.

In our preceding paper we gave the tumor rate and tumor age of the various generations of these hybrids. This cross represents the only case in which the tumor rate and tumor age of the hybrids did not resemble that of either both or one of the parents. The hybrids had a tumor rate of 9% (10%). The tumors belonged to the IV age class.

Without tumors.	With tumors.
326 (79 I 88 II 159 III)	32 (4 I 11 II 17 III)
91% (24% I 27% II 49% III)	9% (12½% I 39% II 48½% III)

The result in this case is difficult to explain. We may have had accidentally to deal with recessives on one or both sides.

(c) English Tan (son of Tumor Mouse 121) + German 73% + 50% (60%). I age class + II age class.

Both parents have here a high tumor rate. The hybrids have also a high tumor rate, but apparently a somewhat lower rate than the English Tan. They belong to the age class of the English Tan (I age class).

Without tumors.	With tumors.
20 (11 I 7 II 2 III)	19 (10 I 7 II 2 III)
51% (55% I 35% II 10% III)	49% (52% I 36% II 12% III)

(d) German + (German + English Tan 143) F_1. The German father was mated to his daughters.

The English female which was mated to the German was Tumor Mouse 143: 50% (60%) + [50% (60%) + 73%]. II age class + I age class.

Without tumors.	With tumors.
2 (2 I) One of these two mice died at the age of 7 mos.	5 (3 I 2 II)

A strain probably rich in tumors and with early tumors.

(e) English + (8 + German) F_4. In this cross two English males were used, one belonging to the substrain English A, the other to the substrain English Sable: 77% (71%) and 63% (74%) + 41% (41%). Total (8 + German) or 51% (53%): (8 + German) F_4. I age class + II age class.

The hybrids have a tumor rate similar to that of the English: 63% (76%). The tumors belong to the I age class. The crosses with both males gave similar results.

Without tumors.	With tumors.
Total: 30 (22 I 7 II 1 III)	52 (27 I 25 II)
37% (74% I 23% II 3% III)	63% (52% I 48% II)
II + III 24%	II + III 76%

In this strain the animals died young.

(f) German + 108. 108 was a white mouse of old Granby stock. She was related to the mice from which the father of the No. 8 strain was derived. 50% (60%) + ? low tumor rate. II age class + (?) age class.

In this case the hybrids had a low tumor rate (8%).

Without tumors.	With tumors.
22 (7 I 10 II 5 III)	2 (1 I 1 (?))
92% (32% I 45% II 23% III)	8%

It is probable that the low tumor rate of 108 prevailed.

(g) German + 6 F_4. No. 6 was a strain in which tumors occurred, but were apparently not very frequent, probably considerably lower than in the German strain. 50% (60%) + ?. II age class + (?) age class.

In this case the F_3 and F_4 generations of the hybrids had less tumors than the F_1 and F_2 generations. In the hybrids the tumor rate was 37% (41%) and the tumors stood somewhere between the II and III age class.

Without tumors.	With tumors.
33 (11 I 10 II 12 III)	19 (4 I 6 II 9 III)
63% (33% I 30% II 37% III)	37% (21% I 32% II 47% III)
II + III 59% III 57%	II + III 41% III 43%

Summary.—If German and English mice are crossed, there is a high tumor rate in the hybrids; both parents have in this case a high tumor rate and the tumors appear on the whole early. In crossing No. 8 with German, the crosses seem to have an intermediate tumor rate. In crossing the (No. 8 + German) with English the high tumor rate of the English prevails and the tumors are early. In crossing German with 108 a very low tumor rate results. It is probable that 108 belonged to a family poor in tumors and that a low tumor rate was dominant. The tumor rate in the crosses of German and 6 F_4 is apparently intermediate between that of the parents. It seems then that in this group there may occur a dominance of the high as well as of the low tumor rate and an intermediate tumor rate may also occur. An unusual result was obtained in the case of the German + Carter in which the tumor rate was considerably lower than in either of the parents, and in this case the tumors appeared also much later than in the parents. This is the only case in which such an abnormality was observed in our experiments.

Summary of Table II.—In hybrids in which the English are one of the parent strains the high tumor rate of the English is dominant in the majority of cases. An intermediate tumor rate prevails in hybrids in which the other parent has a very marked tendency to low tumor rate (Cream, European + 103 European) or where the other parent has a medium rate (⅞ Waltzer ⅛ Cream + No. 8). When the second parent has a very low tumor rate (Cream), the

TABLE II.

Behavior of Various Strains in Hybridization Experiments.

English.	Tumor rate.	Age class.
101 + (European + 103) d_1	i !	L !
English Sable (198) + [(103 + European) F_1 + III daughter of No. 10] d_1	h !	L !
(European 151 + I daughter of No. 10) F_2 + 101 F_2 d_1	c !	h !
English Sable d + (Silver + I daughter of No. 10)	h !	i (?)
English Tan (121) + Cream (3 females)	i !	i !
English Sable (4,444) + Cream d	L !	
8½ + English Sable (4,444) d d_1	h !	h !
European d d_1 + English Tan (daughter of 146, granddaughter of 121)	L (i) !	< L !
Michigan Wild + 101 F_2 d d_1	h (?)	h (?)
(⅞ Waltzer ⅛ Cream + No. 8) d_1 + 101 F_2	i	L ?
English Tan (121) d_1 + German d	L	h
German + (German + English Tan 143) F_1 d d_1.	h	h
English d d_1 + (8 + German) F_4	h !	h !

d indicates dominance of tumor rate of a parent strain.

d_1 indicates dominance of age class of a parent strain.

c indicates tumor rate or age class of parents which is similar.

i indicates a tumor rate or age class intermediate between both parents.

L indicates the tumor rate or age class of the parent with lower tumor rate or late tumor dominant.

h indicates the tumor rate or age class of the parent with the higher tumor rate or early tumors dominant.

! indicates fairly definite results.

? indicates doubtful results.

< indicates that tumors in a cross are later than in either of the parent strains.

low tumor rate may prevail. The age class of the English strain is not to the same extent dominant as their tumor rate.

TABLE III.

European + 103 or 102 (L) (10% to 25%). IV age class.	Tumor rate.	Age class.
1. London + (European + 103) F_2 d d_1	L (c)	L !
2. (European + 102) F_1 d_1 + 8½ F_4	c !	L !
3. 101 + (European + 103) d_1	i !	L !
4. (103 + European) F_1 d d_1 + III daughter of No. 10	L (c) ?	L
5. English Sable (198) d_1 + [(103 + European) F_1 + III daughter of No. 10] d_1	h !	L !

Summary of Table III.—European + 103 (102) is a strain in which the rather low rate did not become entirely recessive in the offspring; the tumor rate of this parent strain was either dominant or the result was intermediate. The tendency of this strain to very

late tumors prevailed in all cases, even when its low tumor rate did not entirely prevail.

TABLE IV.

European.	Tumor rate.	Age class.
European d d_1 + 103, 102	L (?)	L
European d d_1 + English Tan (daughter of 146)	L (i) !	< L !
Cream + European	h (?) !	

Summary of Table IV.—The low tumor rate of the European had a tendency to be dominant with those strains which did not have a very high tumor rate. With the English it was dominant or intermediate; but the higher tumor rate of the European dominated over the lower tumor rate of the Cream.

TABLE V.

European 151.	Tumor rate.	Age class.
European 151 d_1 + 8 F_5 d	h (c) ?	i (L)
European 151 + I daughter of No. 10 d	h !	L
(European 151 + I daughter of No. 10) F_2 + 101 F_2 d_1	c !	h !
European 151 + II daughter of No. 10 d	h !	L ?

Summary of Table V.—The lower tumor rate of European 151 was recessive in crosses whose mother was the first or second daughter of No. 10.

TABLE VI.

No. 8.	Tumor age.	Age class
European 151 + 8 F_5 d	h (c) ?	i (L)
7/8 Waltzer 1/8 Cream + No. 8 d d_1	h !	h
(7/8 Waltzer 1/8 Cream + No. 8) d_1 + 101 F_2	i !	< L (?)
No. 8 F_3 + German	i !	c
English d d_1 + (8 + German) F_4	h !	h !

Summary of Table VI.—The higher tumor rate of No. 8 prevailed probably over the lower tumor rate of European 151; it also prevailed over 7/8 Waltzer 1/8 Cream.

TABLE VII.

I and II daughters of No. 10.	Tumor rate.	Age class.
European 151 + I daughter of No. 10 d d_1	h !	L (?)
(European 151 + I daughter of No. 10) + 101 F_2 d_1	c !	h !
European 151 + II daughter of No. 10 d d_1	h !	L (?)
Silver d + I daughter of No. 10 d_1	i !	L !
Cream d_1 + I daughter of No. 10	i !	L !
8½ + II daughter of No. 10 d	h (i) !	c (L) ?
(8½ + II daughter of No. 10) d + II daughter of No. 10 d_1	L (c)	L

Summary of Table VII.—The high tumor rate of the I and II daughters of No. 10 was dominant over the lower rate of European 151 and No. 8½. It was intermediate with the very low tumor rates of Silver and Cream. Here we find again that various strains with a very low tumor rate depress the dominance of the parent with tendency to high tumor rate more than strains with a tendency to not as low a tumor rate. The independence of the inheritance of the age class from that of the tumor rate is here apparently quite definite.

TABLE VIII.

Cream.	Tumor rate.	Tumor age.
Cream d_1 + I daughter of No. 10	i !	L !
English Tan (121) + Cream (3 females)	i !	i !
English Sable (4,444) + Cream d	L !	
Cream + European d	h (?) !	
⅞ Waltzer ⅛ Cream + No. 8 d	h !	h
(⅞ Waltzer ⅛ Cream + No. 8) d_1 + 101 F_2	i	L (?)

Summary of Table VIII.—The low tumor rate of the Cream is apparently only in one case quite recessive, *viz.*, with European; in other cases it is dominant or the result is intermediate. The age class can be intermediate, when the tumor rate is intermediate, but the lateness of the tumors in the Cream mice can prevail with an intermediate tumor rate.

TABLE IX.

Silver.	Tumor rate.	Tumor age
Silver + I daughter of No. 10 d_1	i !	L !
English Sable + (Silver + I daughter of No. 10) d_1	h !	i (?)

Summary of Table IX.—The Silver strain resembles in its behavior the Cream. With a strain with a high tumor rate it produces crosses with an intermediate tumor rate. The greater lateness again prevails. In a further cross between such an intermediate strain and a strain with a high tumor rate, the high tumor rate of the English dominates.

TABLE X.

No. 8½.	Tumor rate.	Age class.
8½ + II daughter of No. 10 d	h (i) !	c (L) ?
(European + 102) F_1 d_1 + 8½ F_1	c !	L !
(8½ + II daughter of No. 10) d d_1 + II daughter of No. 10	L (c) !	L
8½ + English Sable (4,444) d d_1	h !	h !

Summary of Table X.—The tumor rate of the No. 8½ strain is recessive in combination with a strain with high tumor rate or intermediate crosses result. In these crosses the I age class can also prevail over the later tumors of No. 8½. In combination with (European + 102), however, the late tumor age of the (European + 102) prevails.

TABLE XI.

German.	Tumor rate.	Age class.
No. 8 F_3 + German	i (?)	c !
German + Carter	< L !	< L !
English Tan (121) d_1 + German d	L	h !
German + (German + English Tan 143) F_1	h	h
English d d_1 + (8 + German) F_4	h !	h !
German + 108 (= No. 8 ?) d	L	
German + 6 F_4	i (?)	c (?)

SUMMARY AND CONCLUSIONS.

1. In crossing strains known to differ in their tumor rates, the hybrids show in a considerable number of cases a tumor rate corresponding to the parent with a high tumor incidence; in some cases the offspring have the tumor rate of the parent with the low tumor incidence; in certain cases the tumor rate of the offspring is intermediate between those of the parents.

That these results are not accidental follows from the fact that we could show in some cases that two sisters crossed with the same strains or with the same male give similar offspring, and in other cases we could show that the same individual crossed successively with two strains that behave similarly produces hybrids with a similar tumor incidence.

2. There exists some evidence for the conclusion that different strains in being crossed with other strains differ in their power to impress their tumor rate upon the crosses. Thus the English strain and the I and II daughters of No. 10 have the tendency to transmit to the offspring a high tumor rate, while Cream, Silver, and some European other than 151 have a tendency to transmit a low tumor rate. While crosses of these daughters of No. 10 with European 151 or with No. 8½ show the high tumor rate of the mothers, the crosses of one of the same females with Cream or Silver show an intermediate tumor rate.

3. We find further evidence for our conclusion previously stated

that age class of the tumors and tumor rate are not dependent on the same factor. The age class enters into the crosses as a factor independent of the tumor rate. Thus we find in the crosses between the first daughter of No. 10 and Cream, and in the crosses between the same female and English Silver a similar tumor rate, but the age classes differ in conformity with the difference in the age classes of the parents.

We find, furthermore, that while in some cases a tumor rate and an age class that correspond to each other (high tumor rate, early tumors—low tumor rate, late tumors) are transmitted to the offspring, in other cases tumor rate and age class transmitted to the crosses diverge.

4. It seems that certain strains with very late tumors if mated with strains with earlier tumors have a tendency to transmit to the offspring their own tendency to very late tumors. With a certain strain lateness of the tumors seems to be dominant, while a low tumor rate is not necessarily dominant in the same crosses. This was noticeable in the crosses into which the strain European + 102 or 103 entered as one of the parents.

5. If both parents have a similar tumor rate the offspring have usually a similar tumor rate. There was, however, one exception to this rule in the case of the German + Carter mice, in which the offspring showed a much lower tumor rate and higher age class than either of the parent strains.

THE INFLUENCE UPON THE SPLEEN AND THE THYROID OF THE COMPLETE REMOVAL OF THE EXTERNAL FUNCTION OF THE PANCREAS.

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(*From the Department of Surgical Research of the University of Pennsylvania, Philadelphia.*)

PLATE 77.

(Received for publication, July 2, 1915.)

In the course of studies upon the pancreas in which the external function of the gland was completely removed, either by double ligation of both ducts, cutting and interposing omentum, or by the complete removal of the duodenal portion of the gland, two findings were encountered which seem worthy of a brief communication. The first is that a striking simple atrophy of the spleen rapidly follows such an operation. The second is that the thyroid apparatus of these animals shows a constant change, evidenced macroscopically by a translucency which may amount to an actual transparency, microscopically by an evident increase in the amount of colloid, chemically by a marked increase of the iodine content of the gland, and physiologically by a greatly delayed appearance of tetany, after the complete operative removal of the thyroids and parathyroids.

The details of the operation are shown in Fig. 1. The pancreas of the dog, in our experience, always possesses two ducts, a smaller one draining a definite island of tissue and opening at or near the ampulla of the bile duct, a, and a larger one draining the far greater portion of the pancreas, b. Several authors have described instances in which more than two ducts have been found, so that we have usually tied off the pancreas at A and B, and completely removed the duodenal, duct-bearing portion of the pancreas between the lines A and B.

The results as regards the effects on the spleen and the thyroid

are the same with either method, provided the blocking of the ducts by the interposed omentum has been successful.

Such animals lose weight at first rather rapidly; after several months they reach a level at which they continue or even gain slightly. None of our animals developed sugar in the urine; all showed the voluminous fatty stools typical of loss of pancreatic juice.

Both of the observations here recorded were purely accidental. The operations were undertaken for a definite purpose, and the changes in the spleen and thyroids would doubtless not have attracted our attention had it not been for the extreme changes noted in the first animal of the series. The outline of this animal's spleen (one-half actual size) is shown in Text-fig. 1 (Dog 1). We did not have the measurements of this spleen at the time of the operation upon the pancreas, but if we may judge from the routine measurements of spleens made since, it is fair to assume that this spleen measured at operation at least 18 cm. in length, while at autopsy it measured 6.75 cm.

Following this first observation we repeated the experiment, carefully measuring the spleen at the time of the operation upon the pancreas. The results are seen in Table I and in Text-fig. 1, which shows the simple outline drawings of the spleens of two animals, the outer outline being the spleen at operation, the inner outline the same spleen at autopsy. Under the microscope this atrophy appears as a simple atrophy.

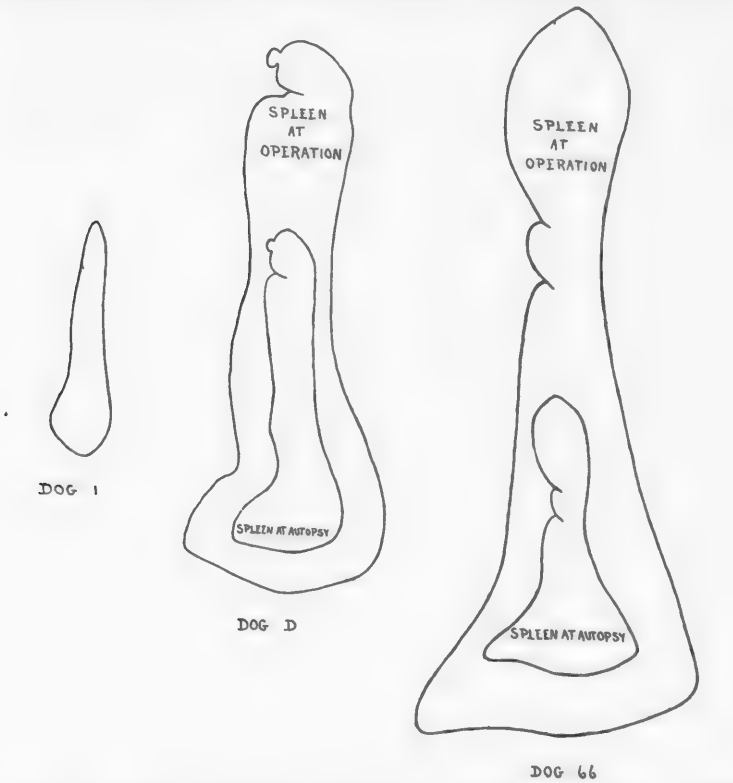
That the splenic atrophy is something more than the expression of the share taken by the spleen in the general loss of body weight is shown, we believe, in the comparative study of some of these animals.

Dog 1 lost 45.6 per cent of its original body weight. While we have not the actual figures, it seems fair to assume that the spleen was at autopsy at least 66 per cent smaller than at the time of the operation. The actual figures for Dog 66 show a loss of weight of 37.9 per cent, with a loss of spleen, as measured by the length of the organ, of 61.9 per cent. Dog 1 reached a constant weight after 150 days, having lost 45.6 per cent of its original weight. Dog 48 was killed at the end of 131 days, having lost 40 per cent of its original weight, but the spleen of Dog 1, the spleen which first

TABLE I.

No. of dog.	Weight at operation.	Weight at autopsy.	Measurements of spleen at operation.	Measurements of spleen at autopsy.	Length of life after operation.	Thyroparathyroidectomy.	Tetany.
	gm.	*gm.*	*cm.*	*cm.*			
1.....	11,400	6,200		Length..... 6.75 Width of head. 1.75 " " tail.. 0.75	Killed after 10 mos.		
A.....	10,080	.....	Length.....18 Width of head. 5 " " tail . 5	Length.....12 Width of head. 3.5 " " tail.. 2.25	" " 21 days		
B.....	8,190	.....	Length.....16 Width of head. 6 " " tail.. 4	Length.....10 Width of head. 3.5 " " tail.. 2.25	" " 12 "		
D.....	10,220	.....	Length.....16 Width of head. 5.5 " " tail.. 2.75	Length..... 9.3 Width of head. 3 " " tail.. 1.5	Died after 3 days of acute pancreatitis		
48.....	9,680	5,805	Length.....18.5 Width of head. 6 " " tail.. 4.5	Length..... 9 Width of head. 3 " " tail.. 2	Killed after 131 days		
24.....	26,122	12,300	Length.....24 Width of head.13 " " tail.. 7	Length.....12.25 Width of head. 6 " " tail.. 3	" " 76 "		
49.....	7,410	4,850	Length.....19 Width of head. 8 " " tail.. 3.5	Length.....11.5 Width of head. 4.5 " " tail.. 2.5	" " 62 "		
66.....	12,580	7,800	Length.....21 Width of head. 8.5 " " tail.. 3.5	Length..... 8 Width of head. 4.5 " " tail.. 1.15	" " 123 "	96 days after pancreatectomy	19 days after removal of thyroids and parathyroids. Recovered.
C.....	9,220	6,600	Length.....18 Width of head. 5 " " tail.. 4	Length.....11.5 Width of head. 3 " " tail.. 2	Died " 49 "	35 days after pancreatectomy	None.
E 25..	13,000	11,630	Length.....20 Width of head. 7.25 " " tail.. 3	Length.....13.5 Width of head. 3.2 " " tail.. 2	Killed " 19 "	7 days after pancreatectomy	Slight tetany. Recovered.

attracted our attention, was only about one-half the size of the spleen of Dog 48, and showed clearly from its slate-colored, shrunken appearance a greater degree of atrophy than did the spleen of Dog 48. This we are inclined to interpret as meaning that the observed atro-



TEXT-FIG. 1. Outline drawings of the spleen before and after the complete removal of the external function of the pancreas.

phy of the spleen had continued in Dog 1 after the body weight had become stationary. That this atrophy is due to something other than the general loss of weight we think is further shown in the dog designated D, which died of an acute pancreatitis three days after operation. The atrophy of the spleen is here clearly marked (Text-

fig. 1), although the subcutaneous and intra-abdominal fat had not appreciably decreased in amount in the short space of three days. The actual weight of the dog at autopsy was not determined.

The observations regarding the thyroid apparatus can be supported in such a variety of ways that their accuracy is hardly to be doubted. The macroscopical appearance of the gland at autopsy is marked by a peculiar translucency which often amounts to an actual transparency. If such a lobe is held towards the light both parathyroids can be plainly seen; in the dog the lower parathyroid body is imbedded completely within the thyroid tissue, and can usually be found only by cutting serial sections; the outline of an opaque object held between the thyroid and the light can be clearly seen through the translucent lobe.

Microscopically the thyroid is seen to contain somewhat more than the normal amount of colloid, with a consequent flattening of the alveolar cells. The parathyroids, as far as we have discovered, are normal; in some instances they have given us the impression of hypertrophy.

In view of the well known variations in the macroscopic and microscopic appearance of the thyroid, one is inclined to speak with reserve concerning conditions based upon such variations. We are therefore fortunate in the case of the thyroid apparatus in having two definite proofs of change, the physiological test and the chemical determination of the specific content of the thyroid.

The physiological results are shown in the three dogs, 66, C, and E 25. Dog 66 developed mild tetanic attacks 19 days after the complete thyroparathyroidectomy. Dog C developed no tetany during the 14 days after the thyroid operation. Dog E 25 developed tetany 7 days after thyroparathyroidectomy.

We have already stated that the parathyroids appear entirely normal, unless, indeed, they are somewhat enlarged. We offer these findings concerning tetany without attempting an explanation. It has been our experience, in several fairly large series of parathyroid tetany experiments, that a normal dog will develop a severe and fatal tetany on the third or fourth day following the complete extirpation of the thyroid apparatus; in a very few instances, not over 5 per cent of our cases, the animal has failed to show any symptoms whatever of tetany.

The iodine determinations in eighteen normal thyroids and in seven thyroids from operated animals are shown in Table II, in which the result is expressed in milligrams of iodine per gram of dry thyroid tissue.

TABLE II.

Source of thyroid.	Weight of dry gland. *mg.*	Iodine per gm. of dry gland. *mg.*
Normal dog	0.2460	0.723
" "	0.1530	0.953
" "	0.4530	1.036
" "	0.5710	1.205
" "	1.0000	1.402
" "	0.3550	1.068
" "	0.1620	0.960
" "	0.7128	2.676
" "	0.4248	1.220
" "	0.1621	1.200
" "	0.42	0.400
" "	0.4	1.050
" "	0.3	0.495
" "	0.7	0.228
" "	0.25	0.800
" "	0.30	0.993
" "	0.2	0.500
" "	0.13	Negative.
Pancreatectomy		
Dog 124	0.5	3.151
" 228	0.25	6.960
" 279	0.4456	1.434
" 257	0.1430	9.925
" 67 P	0.6934	1.134
" 267	0.3502	1.316
" 19 P	0.3448	1.827

Average of 18 normal determinations = 0.9393 mg.
Average of 7 pancreatectomies = 3.678 mg.

We take pleasure in expressing our thanks to Mr. Robert B. Krauss of the Laboratory of the Henry Phipps Institute, for the iodine determinations which were made by the method elaborated by him.[1]

We have found nothing in the literature concerning the thyroid, the pancreas, or the spleen which aids in the explanation of the

[1] Krauss, R. B., The Determination of Iodine in the Presence of Organic Matter, *Jour. Biol. Chem.*, 1915, xxii, 151.

above findings. Any discussion of their meaning at present would therefore be mere speculation, and should, at best, await the confirmation of our results.

SUMMARY.

The complete removal of the function of the pancreas concerned in digestion is followed by marked changes in the spleen and in the thyroid apparatus. Second, the spleen shows an extreme simple atrophy. Third, the thyroid apparatus exhibits a constant change shown by the macroscopic transparency of the gland, by the microscopic increase in the amount of colloid, by the chemical increase of the iodine content of the gland, and by the functional test of the delayed appearance of tetany after the complete removal of the thyroid apparatus.

EXPLANATION OF PLATE 77.

FIG. 1. Diagrammatic outline of the pancreas of the dog (posterior aspect) showing the points at which the operation is carried out. P = pylorus; d = bile duct. For further description, see page 732.

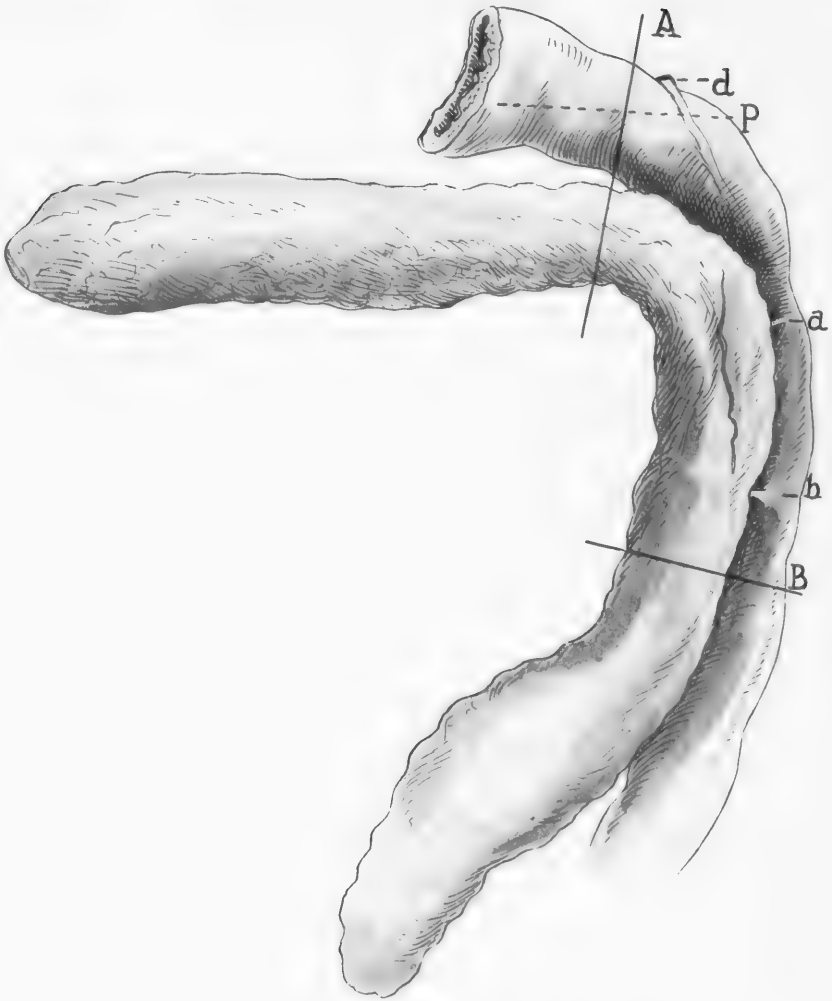


FIG. 1.

(Sweet and Ellis: External Function of Pancreas.)

THE INFLUENCE UPON TADPOLES OF FEEDING DESICCATED THYROID GLAND IN VARIABLE AMOUNTS AND OF VARIABLE IODINE CONTENTS.

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PLATE 78.

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In 1912 and 1914 Gudernatsch[1] reported his studies of the variable effects upon the growth and differentiation of tadpoles produced by feeding different kinds of animal tissues, such as thyroid, thymus, muscle, pancreas, liver, testicle, etc. His most striking findings were that thyroid feeding hastened the differentiation of the tadpoles, at the same time inhibiting their growth, so that he was able to obtain pigmy frogs; and that thymus feeding prevented or delayed their differentiation but favored their growth, so that giant tadpoles resulted. He used fresh tissues, and, in the case of the thyroid, without determining the amount of iodine present.

In view of the known relations of iodine to thyroid activity, it seemed probable that the iodine content of the thyroid fed might also modify its effect on tadpoles. With this in view a supply of tadpoles was brought to the Laboratory on May 9, 1914. These tadpoles were of uniform size, and their age was estimated at about one week.

The stock tadpoles were kept in large granite baking dishes. Those used for experimental observations were kept in small granite basins of about 200 cc. capacity, in which were placed a few small stones. The water in all the basins was completely changed twice

[1] Gudernatsch, J. F., Feeding Experiments on Tadpoles. I. The Influence of Specific Organs Given as Food on Growth and Differentiation, *Arch. f. Entwcklngsmechn. d. Organ.*, 1912–13, xxxv, 457; Feeding Experiments on Tadpoles. II. A Further Contribution to the Knowledge of Organs with Internal Secretion, *Am. Jour. Anat.*, 1913–14, xv, 431.

daily. The experimental basins were kept on tables in the middle of a large room, so that all would be exposed to similar light and temperature conditions. The room temperature was recorded every afternoon. After a few changes by way of trial, it was decided to feed the stock with fresh hog's liver every day, while the experimental animals were fed liver and thyroid on alternate days. The liver was cut up into small pieces, but not crushed. In the earlier experiments the liver was put into the basins in the forenoon and left till late in the afternoon, but this was abandoned because on hot days there was evidence of fermentation or putrefaction which led to the death of some of the tadpoles. The plan of allowing the liver to remain in the basins for one hour and then changing the water eliminated this danger even in the hottest weather. The thyroid was fed in the form of dried powder, in each case the iodine content having been previously determined by Dr. Marine. In the earlier experiments ten tadpoles were placed in each dish, with about 200 cc. of city tap water. Later but five tadpoles were placed in this quantity of water. Beyond taking photographs of the several series, no objective measurements were made of the changes produced.

The questions particularly studied may be summarized as follows: First, the effect upon both growth and differentiation of non-thyroid iodine; *e. g.*, potassium iodide and iodalbin (Parke, Davis & Co.). Second, the effect of thyroid feeding; (a) feeding constant amounts of a series of desiccated thyroids containing progressively increasing percentages of iodine; (b) feeding different quantities of one particular thyroid; (c) feeding thyroid obtained from different species of animals. Third, an attempt to counteract the effect of the thyroid feeding by keeping the tadpoles exposed to cold, by the use of cracker dust, quinine, egg white, egg yolk (both cooked and uncooked), and egg yolk extracted with acetone.

It may at once be stated that the effect of the potassium iodide solutions was negative. As to iodalbin, the results were indefinite. The animals showed early tail absorption, and most of them showed some emaciation at the time of their death; but they all developed some disease resembling a general body edema. Iodalbin contains about 21 per cent of iodine so loosely bound that the toxic effects from free iodine had to be considered, and hence these results can-

not be accepted as suggesting a thyroid-like action for iodalbin. Since these observations were made Morse[2] has published positive results from artificially iodized proteins, and states that the effect is comparable to that produced by thyroid iodine. Further observations must be made before this can be accepted, since there is no conclusive evidence that artificially iodized proteins exhibit an iodothyreoglobulin-like action.

The effect of thyroid feeding was very marked and closely associated with both the iodine content and the amount fed. The details will be exhibited later.

As to factors protecting against the effect of thyroid feeding, only two were found which were certain in their action; namely, exposure to cold and feeding carbohydrate in the form of cracker dust.

We may now examine more in detail the positive results.

The Effect of Thyroid Feeding.

Preparations of desiccated thyroid from human, canine, sheep, and ox glands were used. The human thyroids were obtained from Dr. Crile's clinic and include simple and exophthalmic goitres. All acted alike qualitatively. The sheep and ox glands available were too few to furnish an extended series of experiments, and may be dismissed from present consideration with the statement that there is no reason to believe that, with the material available, one could not get as gradated a series of results as we shall show can be gotten by the use of desiccated dog thyroid. With human glands a gradated series of effects was obtained, but it was not so sharp as with dog's thyroid. This is to be expected because of so many unknown factors in the life history, treatment, etc. Then, too, some of the thyroids had been in 10 per cent formalin for a day or two before desiccation. As regards the effect of formalin one can only state that it does not destroy the thyroid effect.

As examples of the experimental findings, the following protocols are exhibited.

Series I, Dog Thyroid.—Dishes 1, 2, and 3. The tadpoles in this experiment received 50 mg. respectively of three thyroids whose iodine contents were 0.05,

[2] Morse, M., The Effective Principle in Thyroid Accelerating Involutions in Frog Larvae, *Jour. Biol. Chem.*, 1914, xix, 421.

1.40, and 2.92 mg. of iodine per gm. of dried thyroid. This dosage was given every other day, as in all other experiments, unless otherwise indicated. The feeding was started May 16. No liver was fed in this case after the thyroid was started. The tadpoles in Dish 3 were all dead as early as 15 days, and in Dish 2 in 11 days. These were instances of high iodine contents. Four of the tadpoles in Dish 1, receiving thyroid of low iodine content, were living and active as late as Aug. 3—79 days—when the experiment was terminated. These tadpoles were about the size of the controls, but more differentiated, presenting formed (jointed) hind legs. Those in Dishes 2 and 3 died early, were much emaciated, and only slightly differentiated as compared with the controls.

Series I, Dog Thyroid.—Dishes 4, 5, and 6. This experiment is the exact duplicate of the previous one, except that liver was fed on alternate days. The tadpoles in Dish 6 were all dead in 10 days, and in Dish 5 in 2 days,—instances again of early death after feeding with thyroid of high iodine content. Those in Dish 4, getting low iodine thyroid, showed one tadpole still alive and active after 79 days. The tadpoles in this dish developed functional hind legs, were of large size, and had long, well preserved tails. Compared with the controls they showed no emaciation, but marked differentiation. They were larger than those in Dish 1, perhaps due to the liver feeding. In contrast, the tadpoles in Dishes 5 and 6, getting high iodine thyroid, died early, with much emaciation and before there was time for much differentiation. The emaciation was extreme. They literally melted down, the tails rapidly disappearing.

Series I, Dog Thyroid.—Dishes 7, 8, and 9. Here conditions were the same as in the first and second experiments above, except that the thyroid was given only twice. The tadpoles in Dishes 8 and 9, receiving high iodine thyroid, were all dead in 16 and 10 days, respectively, while those in Dish 7, getting low iodine thyroid, were not all dead till 57 days had passed. Those in Dishes 8 and 9 were the more emaciated. Differentiation was not especially affected in any. This experiment shows that only two doses of thyroid of a certain iodine strength will initiate emaciation and lead to early death, the effect being more marked in the case of thyroids with higher iodine contents. Gudernatsch also observed that one feeding with thyroid was sufficient to induce the emaciation and death.

Series II, Dog Thyroid.—In this experiment a series of thyroids containing respectively 0.05, 0.08, 0.18, 0.54, 0.71, and 1.40 mg. (Figs. 1 to 6) of iodine were fed in 50 mg. doses every other day, beginning May 23. Liver was given on alternate days. As early as four days the series as a whole showed a progressive decrease in size and activity in proportion as the iodine percentage increased. Within five days a most remarkable difference was seen, from large active tadpoles in Dish 1, getting the thyroid of lowest iodine content, to markedly emaciated, inert, and highly metamorphosed tadpoles in the dish getting the highest iodine thyroid. At the end of 72 days there was one tadpole living in each of the first three dishes. All were dead in Dishes 4 and 5 within 19 days, and in Dish 6 all were dead within 11 days. The number of days that intervened before the first tadpole died in each dish of the series ran as follows: 8 (accidental), 52, 33, 17, 9, and 5 days. For the second dead in each dish the figures ran: 54, 54, 35, 19, 11, and 7 days. For the third dead: 59, 68, 41, 15, and 11 days. This clearly shows that the death rate parallels the iodine contents. As to differentia-

tion the notes cannot be given in detail, but by way of summary it may be stated that the tadpoles getting high iodine thyroid (Dishes 4, 5, and 6) emaciated so rapidly and died so soon that little differentiation took place. In 41 days the tadpoles in Dish 1 had formed hind legs and were larger than the controls; *i. e.*, they showed marked differentiation together with growth instead of emaciation. On the same date the tadpoles in Dish 2 showed formed hind legs, but were smaller than those in Dish 1, while those in Dish 3 compared in every way with the controls. The final result then seems to be a balance between the tendency to emaciation and to hastened differentiation, and all degrees of differentiation may be associated with all degrees of size. Gudernatsch's more uniform results were undoubtedly due to using thyroid of more constant or high iodine content.

As previously stated, experiments were also made in which the quantity of a particular thyroid fed was varied; *e. g.*, feeding in 10, 20, 30, 40, and 50 mg. doses of some one particular thyroid. The first experiment of this kind to be reported is Series IV, where a dog thyroid containing 1.40 mg. of iodine per gm. of dried gland was fed beginning June 1. The number of days when all were dead ran, respective to the increasing amounts given, 20, 10, 8, 7, 8. Emaciation was very rapid and marked in all, so much so that in the larger doses there was little time for any differentiation. The tadpoles in Dish 1, on the other hand, getting only 10 mg. of thyroid, proceeded within 14 days to the formation of front and hind legs, large frog mouth, and prominent eyes.

Series VII, Dog Thyroid.—Here as in the previous experiment increasing doses were given of a thyroid containing only 0.54 mg. of iodine. Here the number of days within which all were dead were 32, 37, 37, 39, and 42, not very strikingly different. Emaciation was of little consequence in this series, and death was probably largely due to advanced differentiation, which was hastened in all as compared with the controls.

On the whole, the experiments of varying the quantity of thyroid fed are not nearly as clear cut as those where the iodine percentage was varied. I feel, however, that it is only a matter of obtaining a thyroid of suitable iodine content and arranging the quantities fed in a suitably gradated series, in order to get a well gradated series of effects.

The Protective Effect of Feeding Cracker Dust.

Series VIII, Human Thyroid.—In conjunction with some experiments on the possible inhibiting effect of quinine on metabolism, the following experiment was made. One group was fed cracker dust in addition to the regular liver feeding, and the other liver alone. Both groups received 50 mg. of human thyroid with an iodine content of 2.58 mg. per gm. of dried gland, every second day, beginning June 16. Dates of death were of little importance in this case, as they were mostly due to drowning on account of the high degree of differentiation reached. The tadpoles receiving cracker dust became large and acquired functioning hind legs, front legs, and frog-shaped bodies. Those receiving no cracker dust were smaller, had formed hind legs, but no front legs; on the whole they were more nearly like tadpoles, the first more like frogs. Both sets were larger than the controls.

Series XI and XII received every other day dog thyroid with an iodine con-

tent of 1.40 mg. Each series was divided into 5 dishes getting 10, 20, 30, 40, and 50 mg. of thyroid, respectively, beginning June 30. Series XI got cracker dust every second day alternately to the thyroid feeding. Within 8 days the cracker series showed a very distinct progressively increasing emaciation, proportional to the increasing amounts of thyroid fed. This bears out the previous experiments on the effect of variable quantity of thyroid. As early as the sixth day the other group, not getting cracker, showed a marked absorption of tails with decreased activity, while on the seventh day the disparity between the two groups was exceedingly well marked. All the non-cracker group were like small round balls with short conical tails. Owing to the severity of the reaction there was not much difference between the different members of this group. The death dates show a marked shortening of life in the group not receiving cracker. We may conclude then that the feeding of cracker dust delays the tendency of thyroid, when sufficiently active, to hasten death and also tends to prevent emaciation.

The Protective Effect of Exposure to Cold.

Believing that the effects thus far observed were largely due to the well known pharmacological action of thyroid of increasing metabolism, it was thought this action might be lessened by exposing the tadpoles to a lower temperature. Being cold-blooded animals, this would tend to lower their metabolism. With this in mind the following experiments were made.

Series V, Dog Thyroid.—To ten tadpoles kept in a refrigerator were given every second day 50 mg. of a thyroid (dog) containing 1.40 mg. of iodine, which had by previous experiments been shown to have a marked effect. The first dose was given on June 1. All the tadpoles were dead in 27 days, while of the controls kept at room temperature all were dead in 9 days. The controls became markedly emaciated. Those on ice became emaciated toward the end; but earlier, while the controls were still living, they were distinctly larger and less emaciated than the controls. Gradually their tails became absorbed, their bodies smaller, hind and front legs developed along with a frog facies, so that shortly before death they were small but well differentiated; *i. e.*, really pigmy frogs. The controls emaciated so rapidly that there was little differentiation.

Series VIII, Human Thyroid.—These tadpoles were fed cracker dust every second day in addition to the regular liver feeding, and were given every second day 50 mg. of a thyroid containing 2.58 mg. of iodine. Thyroid was started on June 16. One dish was kept on ice, one at room temperature. Of those on ice four were still living at the end of 49 days, when the experiment was terminated, while all of those kept at room temperature were dead in 28 days. Those on ice were of good size, with well preserved tails and slight hind leg buds at the last date of observation, Aug. 3. Those kept at room temperature, compared on the same dates with those on ice, were always larger. Also their differentiation went much further, in that they developed functioning hind legs, front legs, and frog-shaped body and head. And while they died earlier, death was not due to emaciation but to drowning, owing to their complete differentiation.

We may interpret this experiment as follows: At room temperature the stimulation of metabolism by this particular thyroid was not sufficient, in the presence of a more sufficient food supply (cracker), to lead to emaciation, but on the contrary the animals grew large and practically completely differentiated, meeting death by drowning. In the tadpoles kept on ice, metabolism was lowered by the cold so that the tadpoles grew only a little and differentiated only slightly; that is, the stimulating effect of thyroid on metabolism was to some extent counteracted. In the first experiment (Series V) cold protected against the extreme emaciation produced by a certain thyroid at room temperature. In the second experiment (Series VIII) cold tended to counteract the mild stimulation of a certain thyroid which at room temperature led to a high degree of differentiation.

DISCUSSION AND CONCLUSIONS.

We may conclude that the feeding of dried thyroid gland to tadpoles causes an early differentiation in proportion to the quantity fed or the percentage of iodine content of the gland used. With the larger doses and the higher iodine percentages, metabolism is stimulated to such an extent that the animals emaciate rapidly and die early, before there is time for much differentiation. With smaller amounts and lower iodine percentages the size of the animals is roughly inversely proportional to the amount or percentage, so that a close association of differentiation with pigmy size is not characteristic of thyroid feeding as such, as Gudernatsch seems to conclude. One may see early and marked differentiation along with large size. It all seems a question of dosage. The larger sizes are associated with slower differentiation, the smaller sizes with more rapid differentiation, and the smallest sizes may show no differentiation at all, due to the extremely rapid and marked emaciation, and early death. Non-thyroid iodine does not have this effect. The thyroid effect is inhibited by exposure to cold and by cracker feeding. Exposure to cold probably acts by lowering metabolism; cracker feeding, by substituting food other than the animal's own tissues to meet the increased demands caused by the stimulating effect of the thyroid feeding.

Gudernatsch in his earlier paper speaks of the thyroid as stimulating metabolism, which leads to early differentiation and suppresses growth. Later he seems to lean to the view that the thyroid possesses some specific influence on differentiation. It may all be a matter of words, but our present conception is that we are

simply dealing with the well known action of thyroid on metabolism. As the iodine content increases, the thyroid increasingly stimulates the metabolism of the tadpole, which undergoes changes in size, increased growth or rapid emaciation, according to the strength of the action. The tadpole being a larval form, the tissues first to be stimulated to increased metabolism, and later the first to be consumed, are naturally those tissues whose normal function is approaching a normal end, and which, in the normal course of events, are about to undergo metamorphosis. Hastening of differentiation seems then to ensue not as a specific stimulation of differentiation, but only to be the normal result of the stimulation of general metabolism. The seeming specificity of the result lies not in a new action of thyroid, but in its application to a living organism at a specific time in its development.

Most important, of course, is the confirmation of what we may be justified in regarding as an established fact; namely, that the activity and potency of the physiologically active substance of the thyroid is measurable in terms of its percentage iodine content.

Finally, it may be pointed out that the reaction of tadpoles to thyroid feeding is so sensitive that the procedure might well serve as a biological test for the activity of thyroid tissue, superior even to chemical methods.

EXPLANATION OF PLATE 78.

FIGS. 1 TO 6. Photographs of Series II, dog thyroid experiments. Experiment begun May 23 and photographed 7 days later. All were fed 50 mg. of thyroid every other day.

No. 1 received thyroid containing 0.05 mg. of iodine per gm. dried.
" 2 " " " 0.08 " " " " " "
" 3 " " " 0.18 " " " " " "
" 4 " " " 0.54 " " " " " "
" 5 " " " 0.71 " " " " " "
" 6 " " " 1.40 " " " " " "

No change is seen in Nos. 1, 2, and 3 because of the short time interval and the low iodine content of thyroid used, while Nos. 4, 5, and 6 show the characteristic increasing effect of thyroid paralleling the iodine content.

Fig. 1.

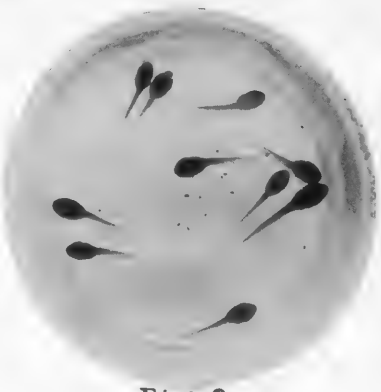

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

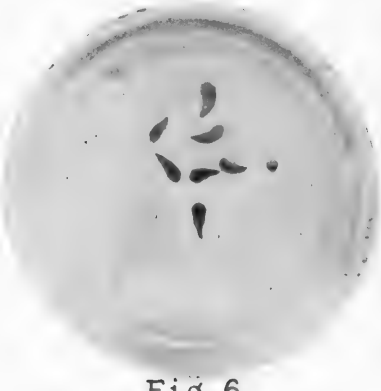

Fig. 6.

(Lenhart: Influence of Desiccated Thyroid Gland upon Tadpoles.)

EXPERIMENTAL PNEUMONIA (FRIEDLÄNDER TYPE).

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Plates 79 to 81.

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INTRODUCTION.

Certain cases of lobar pneumonia in man have for a long time been considered to be caused by *Bacillus mucosus capsulatus* (Friedländer's bacillus). Although this view has been repeatedly questioned, the clinical and pathological studies offer fairly convincing evidence in its support.[1] Few experimental studies on this subject have been undertaken, and little attempt has been made either to reproduce the disease in animals or to study the pathology of the early stages of the condition.

Previous Experimental Work.

Kokawa,[2] working with guinea pigs, mice, and rabbits, was unable to reproduce a definite lobar pneumonia with Friedländer's bacillus. In two instances lesions corresponding more nearly to bronchopneumonia were found. Intratracheal and direct injections of bouillon cultures into the lung were the methods employed. Based upon a very limited number of experiments, Kokawa concluded that the lung could be infected with Friedländer's bacillus and that the infection was aerogenous in origin. Furthermore, he considered that the bacilli were able to cause a pneumonia only when very virulent or when present in great numbers. Certain predisposing factors, according to this author, such as trauma of the lung parenchyma, are necessary for the production of the pathological process. Lamar and Meltzer,[3] working more recently on the subject of experimental pneumonia, have reproduced the disease successfully in two dogs after intrabronchial insufflation of the organism. The pathological findings in this work are not described in detail.

Technique Employed and Results of Experiments.

The following experimental studies are based upon findings in thirty-nine cats. The cats employed for the most part were excep-

[1] Sisson, W. R., and Thompson, C. B., *Am. Jour. Med. Sc.*, 1915, cl, 713.

[2] Kokawa, J., *Ztschr. f. Hyg. u. Infectionskrankh.*, 1905, l, 364.

[3] Lamar, R. V., and Meltzer, S. J., *Jour. Exper. Med.*, 1912, xv, 133.

tionally large, well nourished, well developed animals. They had been kept in confinement for but a very short time and showed no evidence of snuffles or other disease. Each cat was treated as follows:

2 gm. of urethane were given subcutaneously 2 hours before the operation. During insufflation the cat was subjected to a very light ether anesthesia which lasted from 5 to 10 minutes.

In the first 13 experiments, the operation consisted of the following procedures. With the animal on its back, the mouth was held open, the tongue pulled forward and downward. A small filiform catheter was now inserted between the vocal cords and carefully pushed in until fairly definite resistance was encountered, indicating that the tip of the catheter had entered a bronchus. 5 to 8 cc. of a 12 hour culture of *B. mucosus capsulatus* were now insufflated slowly through the catheter with a syringe.

The culture used in this first series of experiments was recovered from the lung exudate of a patient that died 31½ hours after the onset of the first symptoms. The organism had the characteristics of *Bacillus mucosus capsulatus.* 1 cc. of the culture employed, injected intraperitoneally into a medium sized guinea pig, killed the animal in about 18 hours. 38.4 per cent of the cats insufflated with the organism in this manner developed definite lobar pneumonic processes which involved entire lobes or the larger portions of lobes. These cats all yielded positive cultures of *Bacillus mucosus capsulatus* from the heart's blood at autopsy. The remaining animals showed pneumonic processes of various appearances. In some of these, entire lobes were fairly firm, dark red in color, and microscopically showed almost complete atelectasis of the tissue. This was sometimes associated with marked congestion and often with some alveolar exudation. Often, small hemorrhagic areas were found; in others, lesions typical of bronchopneumonia occurred. One of the cats in this series of experiments developed a pericarditis, otherwise no lesions other than the pulmonary ones were found at autopsy. All the animals that showed the lobar type of involvement became extremely prostrated within 10 hours after the insufflation. After 24 hours the animal would lie in a semicomatose condition. The cat was usually killed as shortly before a probable exitus as could be estimated. In no instance did the animal live more than 72 hours. In two instances the cats were found dead at the end of 48 hours. The following experiments[4] will serve to illustrate the course of the

[4] For complete series of experiments see Table I.

disease and the pathological findings in the cats that developed lobar pneumonia after insufflation of cultures of Friedländer's bacillus.

Experiment 3.—Large, well developed, well nourished cat. 7 cc. of culture were insufflated intrabronchially. 24 hours later, the animal was found in a markedly prostrated condition, semicomatose, with respiration definitely accelerated, and gray, slimy, viscid material exuding from the mouth. The cat was killed at this time with chloroform.

Autopsy Findings.—The right inferior lobe is enormously enlarged, being approximately three to four times the size of the corresponding lobe on the left. The visceral pleura over the enlarged lobe is cloudy, but smooth. The left lung weighs 9.5 gm., the right 46 gm. (The normal weights are about 9 and 13 gm. respectively.) The right lower lobe is firm and consolidated except for narrow marginal areas where alveolar structure can be made out. The color is in general dark red and the lobular markings are accentuated because of injection of the entire lobular lymphatics. On cross-section the cut surface is covered with a large amount of stringy, mucoid, slightly sanguinous, grayish material. On pressure, this can be easily expressed from numerous foci. The surface, on removal of the exudate, presents a very striking, mottled, marble-like appearance. Poorly defined, gray areas are surrounded by hemorrhagic areas. No clearly defined lobular distribution of the process can be made out. Pieces of the lung sink in water. The tissue does not present a granular appearance. Over the surface of the left superior lobe, about the hilus, are raised (2 to 5 mm. in diameter), injected, nodular areas. These, on cross-section, represent small, grayish, circumscribed foci of consolidation with hemorrhagic margins. The findings elsewhere show nothing remarkable.

Cultures taken from the heart's blood, lung exudate, and spleen all showed a pure culture of *Bacillus mucosus capsulatus.*

Microscopic Findings.—Sections were taken from various portions of the right inferior lobe and fixed in formalin and Zenker's fluid, stained in hematoxylin and eosin, methylene blue, and also by Gram's method. All the sections show alveolar spaces completely filled with exudation except in tissue taken from the margin of the lung. Here the demarcation between air-containing and infiltrated lung tissue often is very sharply shown. In most of the sections, consolidation is complete and does not suggest a focal or bronchopneumonia. The alveolar exudate consists chiefly of dense blue-staining masses of extracellular, Gram-negative, capsulated bacilli and polymorphonuclear cells (Fig. 3). Scattered throughout the field are spaces almost completely filled with red blood cells. Large vacuolated mononuclear cells are rarely seen. Some of the bronchi are completely filled with exudate, others are air-containing.

Sections taken from the focal lesions in the left lung show alveolar spaces about bronchi that are filled with exudate similar to that described above.

Experiment 5.—Large adult, male cat. Technique the same as in the previous experiment. 24 hours after insufflation the animal is markedly prostrated, refuses food, is dyspneic, and coughs. Killed 28 hours after insufflation.

Autopsy Findings.—The pleural cavities contain no free fluid; the visceral pleura of the right inferior lobe is somewhat opaque. The left lung weighs 21 gm., the right 51 gm. The right inferior lobe is greatly enlarged, also the adjoin-

ing middle lobe of this lung. Both are very much redder than normal and in places suggest infarcted lung tissue. The consistence is firm, non-crepitant, except for marginal areas. On cross-section the surface is homogeneous in appearance, and moist. A small amount of seropurulent, slightly sanguinous exudate can be expressed from the bronchi. This is not viscid in character. The scraped surface has a slightly granular appearance.

Cultures taken from the heart's blood and from the lung exudate show pure growth of characteristic capsulated bacilli.

Microscopical Findings.—Sections taken from various portions of the consolidated lung were fixed in Zenker's fluid and formalin and stained with methylene blue and eosin and by Gram's method. All the alveoli are completely filled with polymorphonuclear cells. Scattered through the exudate are rare vacuolated, large mononuclear cells, few capsulated bacilli, a small amount of fibrin, and few red blood cells. The capillaries of septal walls are slightly dilated. The lumina of the bronchi contain granular coagulum and polymorphonuclear leucocytes.

Experiment 6.—Large, well nourished, adult cat. The technique employed was the same as in the last two experiments. The animal 24 hours after insufflation is inactive, refuses food, and seems somewhat dyspneic. 48 hours after injection dyspnea is more marked; found in semicomatose condition. Killed at this time.

Autopsy Findings.—The left lung weighs 12 gm., the right 42 gm. The pleural cavities contain no free fluid. The visceral pleura of the right inferior lobe is somewhat opaque. This lobe is greatly enlarged. The entire lobe is apparently completely consolidated and is moderately firm in consistence. On cross-section the surface presents an almost homogeneous appearance. It is pink to light red in color. A small amount of mucopurulent exudate can be scraped off with the edge of the knife and expressed from the bronchi. Cultures from the heart's blood and the lung exudate show a pure growth of capsulated bacilli.

Microscopical Findings.—Sections were taken from the consolidated lobe, fixed, and stained as in the previous experiments. They show alveolar spaces uniformly and completely filled with exudate. This everywhere has the same characteristics, and consists chiefly of polymorphonuclear cells and few large phagocytic mononuclear cells, often containing capsulated Gram-negative bacilli. There are a few red blood cells and little fibrin present. The bronchi often contain a similar exudate.

The second method employed to reproduce the Friedländer type of pneumonia was the direct injection of bouillon cultures into the femoral vein. Four animals received 5 cc. of a culture similar to that used in the preceding series. These showed evidence of slight toxemia within twenty-four hours after the injection. The clinical picture was quite different from that in the preceding experiments and it is believed that all the animals would have survived. They were killed from one to five days after the injection. The autopsies failed to show any pulmonary lesions.

A third series of ten animals were treated as follows: The ether anesthesia was followed by an intrabronchial insufflation of strong ammonia fumes for a period of thirty seconds to one minute. In this connection it should be stated that the injury to the lung parenchyma produced by this procedure was not extensive. In the majority of animals no lesions or only slight hemorrhagic foci were found at autopsy after the insufflation of ammonia fumes. The fumes were forced in through a catheter by blowing through a series of bottles arranged with two-hole stoppers. In most instances the fumes elicited definite coughing. Immediately after the insufflation of the ammonia, 5 cc. of a 12 hour bouillon culture of *Bacillus mucosus capsulatus* were injected into the femoral vein. 30 per cent of these cats developed pulmonary lesions that had the characteristics of lobar pneumonia. In each of these cases the animals developed symptoms quite similar to those in the first series that showed lobar pneumonic processes. The extreme prostration was striking. The animals in this last series that developed no pulmonary lesions acted like those injected intravenously without intrabronchial insufflation. The autopsy findings were also similar.

Experiment 30 will serve to illustrate the course of the disease and the pathological findings in a cat developing a lobar pneumonia 56 hours after the intravenous injection of the organism and intrabronchial insufflation of ammonia fumes.

Experiment 26.—Large, well nourished, and well developed cat. 24 hours after injection the animal is markedly prostrated, refuses food, and is slightly dyspneic. After 48 hours it is in a semicomatose condition with marked dyspnea. It attempts to hold up its head as if to facilitate respiration. It was killed at this time.

Autopsy Findings.—The pleural cavities contain no free fluid. The visceral pleura of the right inferior lobe is distinctly opaque and is covered over limited areas by a thin deposit of fibrin. There is extreme enlargement of this lobe; it is firm, non-crepitant, and apparently completely consolidated. The right lung together with the heart was preserved in Kaiserling's solution as a permanent specimen (Fig. 1). This weighs 110 gm. (normal weight 30 to 35 gm.). The inferior lobe is dark red in color and shows poor definition of lobules with absence of alveolar markings except along the margins. The cut surface of the consolidated lobe (Kaiserling preparation) presents a non-homogeneous appearance, but apparently is diffusely infiltrated (Fig. 2). Large hemorrhagic, poorly defined areas in the central portion are everywhere surrounded by gray, slightly hemorrhagic tissue. The process does not suggest a lobular distribution.

Culture taken from the heart's blood shows no growth after 48 hours.

Microscopical Examination.—(Kaiserling material.) Although the sections are not entirely satisfactory, the alveolar spaces are found to be filled with an exudate made up of polymorphonuclear cells, many capsulated bacilli, considerable coarse fibrin, many red blood cells, and a few large vacuolated mononuclear phagocytic cells. The amount of exudate varies somewhat, however; nowhere are air-containing alveoli seen. The bronchi often contain an exudate similar to that in the alveoli. The bronchial epithelium is everywhere intact.

TABLE I.

Series.	Experiment No.	Insufflation.	Intravenous injection.	Pneumonia, lobar.	Blood culture.	Duration.
						hrs.
I	1	*B. mucosus capsulatus*		0	0	
	2	"		0	0	
	3	"		+	+	24
	4	"		0	Streptococci	
	5	"		+	+	28
	6	"		+	+	48
	7	"		+	+	27
	8	"		+	+	36
	9	"		0	0	
	10	"		0	Streptococci	
	11	"		0		
	12	"		0	"	
	13	"		0	"	
II	14		*B. mucosus capsulatus*	0	0	
	15		"	0	0	
	16		"	0	0	
	17		"	0	0	
III	18	Ammonia fumes	"	+	+	36
	19	"	"	0	0	
	20	"	"	0	0	
	21	"	"	+	+	72
	22	"	"	0		
	23	"	"	0	0	
	24	"	"	0	+after 30 hrs.	
	25	"	"	0	0	
	26	"	"	++	0	56
	27	"	"	0	0	
IV	28	"	Pneumococci (Group 1)	0	0	
	29	"	"	+	0	72
	30	"	"	0		
	31	"	Streptococci	0	0	
	32	"	"	0	0	
	33	"	"	0	0	
	34	"	"	0	+	
	35	"	Pneumococci (Group 4)	0	0	
	36	"	"	0	0	
	37	"	"	0	0	
	38	"	"	0	0	

In a fourth series of experiments, various strains of streptococci and pneumococci were injected intravenously after intrabronchial in-

sufflation of ammonia fumes, as in the previous series. The organisms were obtained either from lung punctures or from the lung tissue of patients dying of lobar pneumonia. Eleven cats were each given 5 cc. of a 24 hour bouillon or hydrocele fluid culture of the various organisms. But one of the animals developed pulmonary lesions. In Experiment 29 after the injection of a culture of pneumococci (Group 1) a very early lobar pneumonia was found.

SUMMARY.

The foregoing experiments show that in cats a definite lobar pneumonia may be caused by *Bacillus mucosus capsulatus*. Judging both from the clinical course and from the pathological findings, this form of pulmonary infection differs from the usual pneumococcus types of pneumonia and closely resembles the so called Friedländer's bacillus or *Bacillus pneumoniæ* in man. In all instances in which a lobar pneumonia was found after the injection of the bacillus, a similar organism was recovered from the lung, and in no case was this associated with other organisms. The course of the disease in cats is very short, the animals developing early symptoms of profound toxemia. In 87 per cent of the animals showing a lobar pneumonia positive blood cultures were obtained. The pathological findings, judging from the early stages of the disease, are subject to considerable variation. In some instances the process may suggest a pseudolobar or confluent lobular distribution. In these cases the lung has a mottled, marble-like appearance. In the majority of cases, however, the process gave a more homogeneous appearance, suggesting a diffuse and uniform distribution. Foci of hemorrhage were not uncommon in both. Such areas cause the mottled appearance sometimes found. In all instances the consolidated lung presents a greater infiltration of tissue than is usually seen in other types of experimental pneumonia. Although the exudate as seen on the cut surface may be abundant and especially viscid in character, this is not present in most cases. The cut surface of the consolidated lung does not present a granular appearance. The histological findings are also subject to considerable variation. In most instances the infundibular and alveolar spaces are completely filled with an exudate made up chiefly of polymorphonuclear cells. Associated with

these are the capsulated bacilli, large vacuolated mononuclear phagocytic cells, and red blood cells, and occasionally small amounts of fibrin. The organisms may vary greatly in numbers. Some sections show spaces almost completely filled with bacilli. The contrast between spaces containing an exudate consisting chiefly of polymorphonuclear forms and an adjoining one filled with organisms is often striking (Fig. 4). The bacilli found are both intra- and extracellular.

The large vacuolated cells are numerous in this type of pneumonia. They apparently are the first cells to become phagocytic. Often they are seen to contain as many as 10 to 15 capsulated bacilli, while polymorphonuclear cells in the same exudate contain no organisms. The histogenesis of these cells seems to be somewhat clearer from the study of these early stages of pneumonia. In many instances one sees swollen, partially desquamated epithelial cells along the alveolar wall. These closely resemble the large vacuolated forms. Various types of these vacuolated mononuclear cells were observed. These may well represent stages of development from the desquamating epithelial cell to the large vacuolated form. Although similar cells may arise elsewhere, we have been led to regard them in our studies as epithelial in origin (Fig. 5).

The number of red blood cells and the amount of fibrin present in the exudate vary greatly. Small foci consisting of alveolar spaces filled with erythrocytes are not uncommon. The fibrin is very much less abundant than in most types of pneumonia.

From the above experiments it is seen that a lobar pneumonia in cats can be produced at least by two methods, either by intrabronchial insufflation of the organism or by direct injection into the veins, provided that in the latter case an irritant is introduced into the lungs. In each case there is little doubt but that a local injury of the lung parenchyma was produced. Without this injury (that is, by intravenous injection of the organism alone), no pulmonary lesion was obtained.

Further studies with both these methods must be undertaken to ascertain more exactly the sequence of the pathological process. It seems probable that they are identical in each case.

The results obtained from the second method employed to produce

a lobar pneumonia offer suggestive evidence in support of a hematogenous causation of this disease in at least certain instances. It is not proposed to discuss the aerogenous versus hematogenous theories at this time. Kidd[5] has recently reviewed the subject and states that the aerogenous theory for the causation of pneumonia is most widely held. This view has gained credence especially since the work of Meltzer and Lamar. In spite of this, Kidd emphasizes the fact that based on our knowledge of pulmonary infections in man and upon theoretical grounds and upon certain experimental facts, the hematogenous theory seems more plausible.

No definite conclusions can be drawn from the last series of experiments. From this limited study it seems probable that lobar pneumonic processes are produced less easily after intravenous injection of various cocci and insufflation of irritating substances than by similar treatment with Friedländer's bacillus.

CONCLUSIONS.

1. A typical lobar pneumonia can be produced in cats either by intrabronchial insufflation of *Bacillus mucosus capsulatus* (Friedländer's bacillus) or by its intravenous injection, provided that a local injury of the lungs coexists.

2. Pathologically, this form of experimental pneumonia, although subject to considerable variation, has distinctive characteristics.

The authors wish to take this opportunity to thank Dr. W. T. Councilman and Dr. H. A. Christian for valuable suggestions in connection with this work; and Mr. H. S. Aiken for the accompanying illustrations.

EXPLANATION OF PLATES.

PLATE 79.

FIG. 1. Photograph of right lung (Experiment 26), lateroposterior view, showing markedly enlarged consolidated inferior lobe (about actual size).

FIG. 2. Cross-section of lung and heart (Experiment 26), as seen in Fig. 1, showing complete consolidation of inferior right lobe (about actual size).

PLATE 80.

FIG. 3. Section from consolidated lung (Experiment 3), showing the character of the exudate (low power). A, capsulated bacilli; B, polymorphonuclear cells; C, fibrin; D, dilated capillary of alveolar wall.

[5] Kidd, P., *Lancet*, 1912, i, 1590.

FIG. 4. Section from consolidated lung (Experiment 3), showing the difference in the character of the exudate in adjoining alveoli (high dry). A, numerous capsulated bacilli; B, polymorphonuclear forms.

PLATE 81.

FIG. 5. Section from consolidated lung (schematic reproduction) (Experiment 8), showing vacuolated type of cells (oil immersion). A, epithelial cell of alveolar wall; B, early forms of desquamated epithelial cells, showing vacuoles; C, typical large vacuolated cells containing capsulated bacilli and pigment; D, polymorphonuclear cells containing capsulated bacilli.

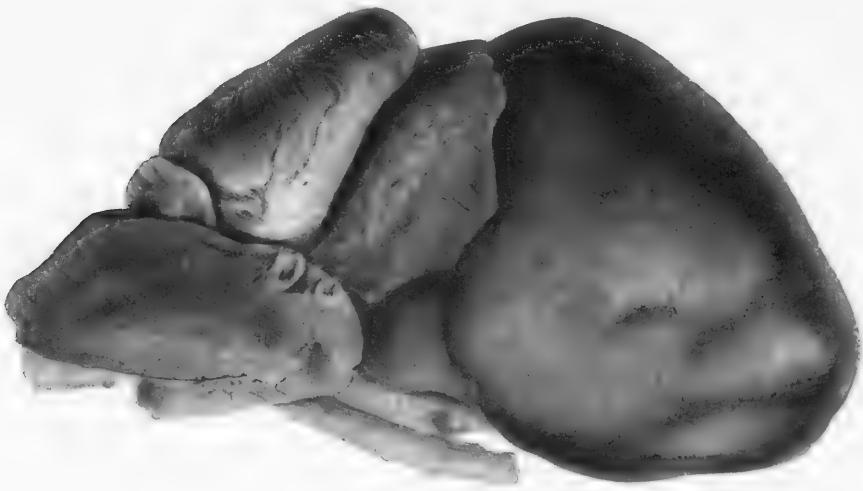

FIG. 1.

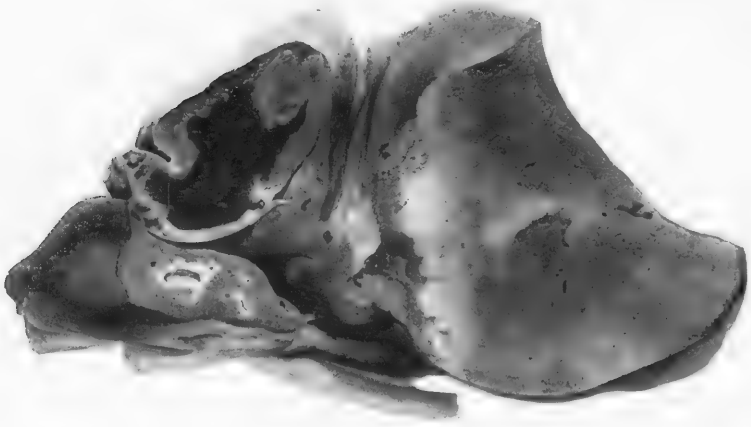

FIG. 2.

(Sisson and Walker: Experimental Pneumonia.)

FIG. 3.

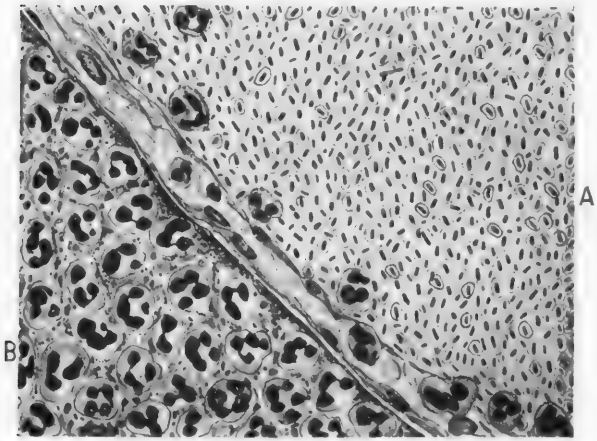

FIG. 4.

(Sisson and Walker: Experimental Pneumonia.)

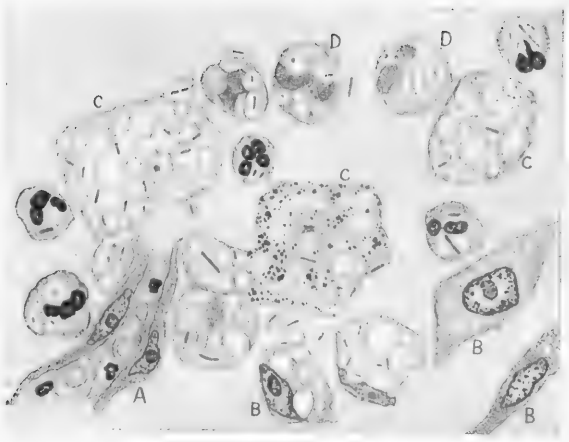

FIG. 5.

(Sisson and Walker: Experimental Pneumonia.)

AN EXPERIMENTAL STUDY OF BLOOD GLYCOLYSIS. THE EFFECTS OF THYROID AND ADRENAL EXTRACTS AND PHLORHIZIN ON GLYCOLYSIS IN VITRO.

By GEORGE M. MACKENZIE, M.D.

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(Received for publication, June 17, 1915.)

Efforts to localize and measure glycolysis have been very unsatisfactory, especially since the theory of Cohnheim[1] concerning glucose destruction by the combined action of extracts of muscle and pancreas has been shattered by Levene and Meyer.[2] Levene and Meyer showed that the sugar was not really oxidized in such mixtures, but converted into other forms which fail to reduce copper. Hydrolysis of the resulting fluid restores the glucose with its original power of reduction. Nevertheless, Levene and Meyer admitted that glycolysis could be brought about by leucocytes, and many others have shown that if blood be incubated for several hours the amount of glucose remaining is distinctly less than in the freshly shed blood. This oxidation of glucose in the blood is probably, however, of very slight physiological importance.

It is known that glycosuria appears after the injection of certain materials into the body, and it was thought desirable to determine, if it could be demonstrated by using the ordinary blood glycolysis as a test, whether these substances have any effect upon the action of a sugar-splitting enzyme. It was realized that other explanations of their action are generally accepted and that their application to a somewhat artificial process of glycolysis outside of the body might be criticized. Nevertheless, the experiments were made to learn whether, in any degree, they could act to inhibit a glycolytic process.

[1] Cohnheim, O., *Ztschr. f. physiol. Chem.*, 1904, xlii, 401; 1906, xlvii, 253.
[2] Levene, P. A., and Meyer, G. M., *Jour. Biol. Chem.*, 1911, ix, 97.

EXPERIMENTAL.

Study of Blood Glycolysis. Effects of Thyroid and Adrenal Extracts and Phlorhizin on Glycolysis in Vitro.

Method.

Dogs were used in all the experiments. The animals were etherized and either a carotid or femoral artery exposed. A cannula, with rubber tube attached, was inserted in the artery and the blood drawn directly into a sterile bottle containing glass beads. The blood was defibrinated and 20 cc. portions pipetted off and placed in sterile 150 cc. Erlenmeyer flasks which were plugged with sterile cotton. The amount of reducing substance in the blood drawn was determined immediately; in most of the experiments duplicate portions were used. Table III shows the difference found in the duplicate portions of the same blood. The other portions, after the substances being studied for their effect on glycolysis had been added, were placed in the incubator at 38° C. for varying periods of time and then the reducing substance determined. For comparison of the rate of glycolysis an untreated portion of the same blood was, in each case, incubated along with the treated portions.

In all the determinations the protein was precipitated with colloidal ferric hydroxide and sodium sulphate[3] and the reducing substance found by the method of Bertrand.[4]

Inasmuch as only comparative values were sought rather than an accurate determination of the normal blood sugar level of the dog, no effort was made to avoid the effects of excitement, struggling, and ether. The figures, therefore, are somewhat higher than those of Shaffer,[5] Embden, Lüthje, and Liefmann,[6] who eliminated these factors as completely as possible. Shaffer's results varied between 0.02 and 0.065 per cent, Embden, Lüthje, and Liefmann's between 0.057 and 0.088 per cent. Macleod[7] gives the figures of several observers which vary between 0.086 and 0.207 per cent. His own vary between 0.079 and 0.205 per cent.

[3] Rona, P., and Michaelis, L., *Biochem. Ztschr.*, 1908, vii, 329. Macleod, J. J. R., Diabetes, London, 1913, 28.

[4] Bertrand, G., *Bull. de la Soc. chim. de Paris*, 1906, xxxv, 1285.

[5] Shaffer, P. A., *Jour. Biol. Chem.*, 1914, xix, 297.

[6] Embden, G., Lüthje, H., and Liefmann, E., *Beitr. z. chem. Phys. u. Path.*, 1907, x, 265.

[7] Macleod, *loc. cit.; Jour. Biol. Chem.*, 1913, xv, 497.

In all the experiments sterile conditions were maintained as far as possible. Cultures were taken from the incubated blood in the first few experiments. A few of these were contaminated but most of them were sterile. To determine the effect of contamination upon the rate of disappearance of reducing substance, duplicate portions of the same blood were incubated for the same period of time, one under aseptic conditions, the other without sterile glassware and without any effort to avoid contamination. The results are shown in Table I. The loss of reducing substance is tabulated here, and in other tables that follow, as percentile glycolysis, which expresses the ratio of the amount of sugar lost during incubation to the amount originally present; *i.e.*, $\frac{\text{sugar lost per 100 cc.}}{\text{sugar originally present per 100 cc.}} =$ percentile glycolysis.

TABLE I.

Comparative Values for Blood Glycolysis under Sterile and Non-Sterile Conditions.

Experiment.	Glucose before incubation.	Condition of blood during incubation.	Duration of incubation.	Glucose after incubation.	Percentile glycolysis.
	per cent		*hrs.*	*per cent*	
I	0.130	Sterile	3	0.073	43.8
		Non-sterile	3	0.076	41.5
II	0.130	Sterile	3	0.075	42.3
		Non-sterile	3	0.073	43.8
III	0.100	Sterile	3	0.074	26.0
		Non-sterile	3	0.075	25.0

From this it appears that the difference in the amount of glycolysis taking place in sterile and contaminated blood during a period of three hours' incubation is within the limits of experimental error, and, therefore, that failure to maintain sterile conditions probably has little or no effect on the amount of glycolysis occurring within a period of three hours.

In order to determine how much variation there might be in the rate of glycolysis in several portions of the same blood taken at the same time and kept under the same conditions, three 20 cc. portions of the same blood were incubated in separate flasks and the percentage of sugar then determined. The results are shown in Table II.

TABLE II.

Variations in the Amount of Glycolysis in Several Portions of the Same Blood.

Experiment.	Glucose before incubation.	Portions.	Duration of incubation.	Glucose after incubation.	Percentile glycolysis.
	per cent		*hrs.*	*per cent*	
I	0.100	*A*	3	0.069	31.0
		B	3	0.074	26.0
		C	3	0.073	27.0
II	0.130	*A*	3	0.070	46.1
		B	3	0.073	43.8
		C	3	0.075	42.3

This indicates that different portions of the same blood under identical conditions show very slight variation in the rate of glycolysis, and this factor, therefore, does not explain irregularities in the results after adrenalin, thyroid extract, and phlorhizin have been added to the blood.

Table III is given to show the amount of experimental error by the methods used. The greatest difference found was about 3 per cent.

TABLE III.

Percentage of Blood Sugar in Two Portions of the Same Blood.

	Portions.	1	2	3	4	5	6	7	8
Percentage of blood sugar	*A*	0.110	0.099	0.174	0.176	0.100	0.175	0.131	0.133
	B	0.112	0.096	0.172	0.175	0.101	0.173	0.129	0.135

Effect of Fresh Thyroid Extract upon Blood Glycolysis.

The results of experimental work upon the relation of the thyroid secretion to carbohydrate metabolism are somewhat conflicting. Macleod[8] after reviewing the work that has been done concludes that the thyroid glands, in so far as carbohydrate metabolism is concerned, have no direct influence whatever. The results which some observers[9] have obtained, tending to show that the thyroid furnishes a substance which lowers the assimilation limit for dextrose, he ascribes to parathyroid effect.

Clinical observations on the blood sugar, glycosuria, and dextrose assimilation limit in cases of hyperthyroidism and hypothyroidism

[8] Macleod, Diabetes, London, 1913, 104.

[9] Eppinger, H., Falta, W., and Rudinger, C., *Wien. klin. Wchnschr.*, 1908, xxi, 241; *Ztschr. f. klin. Med.*, 1908, lxvi, 1; 1909, lxvii, 380.

seem to indicate, however, that the gland does have some effect upon carbohydrate metabolism. When the thyroid is supplying the blood with abnormally large quantities of its secretion, the blood sugar rises, glycosuria often appears, and the dextrose assimilation limit is lowered. The reverse is the case in myxedema. If the thyroid secretion could be shown to contain an antiferment it might act simply by preventing the sugar-splitting ferments of the blood and tissues from oxidizing the carbohydrate. This would result in hyperglycemia, glycosuria, and lowered tolerance for glucose. The following four experiments were done to determine whether such an antiferment could be extracted from the gland.

Thyroid extract was prepared by extirpating the entire gland of a dog, and grinding it up in a sterile mortar with sterile normal salt solution or sterile Ringer solution, care being taken to exclude the parathyroids in so far as possible. The amount of extract used, whether 5 or 10 cc., represented one entire gland. The extract was added to the blood taken from another animal and the mixture incubated. Controls with the amounts of Ringer solution or saline used in the extract were incubated at the same time. For comparison one portion of untreated blood was incubated for the same period. The results are shown in Table IV.

TABLE IV.

Experiment.	Glucose before incubation.	Mixtures incubated.	Duration of incubation.	Blood sugar after incubation.	Percentile glycolysis.
	per cent		*hrs.*	*per cent*	
IX	0.098	Blood 20 cc. + 10 cc. fresh thyroid extract	3	0.060	39.7
		" " " " " " " Ringer solution	3	0.054	44.8
		" " " untreated	3	0.080	18.4
X	0.173	" " " + 5 cc. fresh thyroid extract	3⅓	0.096	44.5
		" " " " " " " Ringer solution	3⅓	0.106	38.7
		" " " untreated	3⅓	0.119	31.1
XI	0.175	" " " + 5 cc. fresh thyroid extract	2½	0.132	24.6
		" " " " " " " Ringer solution	2½	0.138	21.1
		" " " untreated	2½	0.145	17.1
XII	0.152	" " " + 5 cc. fresh thyroid extract	3	0.094	38.1
		" " " " " " " 0.9 per cent sodium chloride	3	0.088	42.1
		Blood 20 cc. untreated	3	0.103	32.2

From these results one cannot conclude that the thyroid secretion contains any substance which materially affects the rate of glycolysis *in vitro*. In each case there is apparently some acceleration of

glycolysis both in the flask containing the extract and in the flask containing the Ringer or physiological salt solution. In Experiment IX the acceleration is well marked, in the others, slight. Possibly this is due simply to the dilution, because in the experiment in which the glycolysis was most marked the dilution was greatest. The experiments do not, of course, settle the question of whether or not the thyroid gland furnishes an antiferment which inhibits glycolysis; it is possible that the gland contains such a substance but that it is not extracted by the methods used. To the extent, however, that the conditions of glycolysis *in vitro* are similar to glycolysis in circulating blood, they furnish an argument against the hypothesis that the thyroid gland affects carbohydrate metabolism by inhibiting ferment action.

Effect of Adrenalin on Blood Glycolysis.

Blum,[10] fourteen years ago, showed that subcutaneous or intravenous administration of adrenal extract causes glycosuria. Since then this work has been abundantly confirmed, and it has been shown that adrenalin regularly causes a hyperglycemia. Experiments of Pollak[11] showed that adrenalin produces glycosuria even after the resection of the splanchnic nerve, while the work of Claude Bernard[12] and Eckhard demonstrated that, after such resection, puncture of the floor of the fourth ventricle is not followed by glycosuria. The adrenalin is, therefore, thought to act by stimulating the peripheral sympathetic nerve endings in the liver. Moreover, it causes the hyperglycemia even in animals whose livers have been rendered glycogen-free by starvation. This is thought to be due to the production of sugar from protein. Despite these explanations of the action of adrenalin in the production of hyperglycemia and glycosuria, it seemed to be worth while to determine whether or not the adrenal secretions contain a substance acting as an antiferment. With this possibility in mind the following experiments were done.

[10] Blum, F., *Deutsch. Arch. f. klin. Med.*, 1901, lxxi, 146.

[11] Pollak, L., *Arch. f. exper. Path. u. Pharmakol.*, 1909, lxi, 149.

[12] Bernard, C., Leçons sur la physiologie et la pathologie du système nerveux, Paris, 1858.

Parke, Davis and Co. adrenalin was used. Quantities varying from 1 to 5 cc. were added to 20 cc. portions of blood and the mixtures incubated for varying periods. The sugar content of the blood was determined immediately after drawing the blood, and in the incubated portions immediately after the expiration of the period of incubation. A portion of untreated blood was also incubated for comparison. The results are shown in Table V.

TABLE V.

Experiment No.	Adrenalin 1:1,000.	Glucose before incubation.	Duration of incubation at 38° C.	Glucose after incubation.	Percentile glycolysis.	Glucose in untreated blood after incubation.	Percentile glycolysis in untreated blood.
	cc.	*per cent*	*hrs.*	*per cent*		*per cent*	
IX	5	0.098	3	0.039	60.2	0.080	18.3
X	5	0.173	3⅓	0.102	41.0	0.119	31.1
XI	5	0.175	2½	0.150	14.2	0.145	17.1
XIII	5	0.152	3	0.064	57.8	0.103	32.2
XIV	1	0.100	2½	0.049	51.0	0.037	63.0
XIV	2	0.100	2½	0.043	57.0	0.037	63.0
XIV	3	0.100	2½	0.044	56.0	0.037	63.0
XV	2	0.174	5	0.124	28.8	0.120	31.0

In Experiments IX and XIII the blood with added adrenalin showed a definite acceleration of glycolysis as compared with the untreated blood. In Experiment IX about three times as much reducing substance disappeared from the adrenalin blood as from the untreated blood, in Experiment XIII a little less than twice as much. In the other experiments the difference in the percentile glycolysis between the treated and the untreated blood was so slight as to be almost within the limits of experimental error.

The results are so irregular that one is not justified in concluding that adrenalin contains any substance which either accelerates or inhibits blood glycolysis. The explanation of the apparent acceleration in two experiments is not clear.

Effect of Phlorhizin on Blood Glycolysis.

It is believed that the glycosuria after administration of phlorhizin[13] results from the power of phlorhizin to render the kidney more permeable to sugar in the blood, thus lowering the threshold for sugar excretion. There is usually a hypoglycemia. Beyond the fact that phlorhizin renders the kidney more permeable to

[13] For a full discussion and complete bibliography of phlorhizin, see Lusk, G., *Ergebn. d. Physiol.*, 1912, xii, 315.

glucose, little is known of its action. The possibility of its acting as an antiferment suggested itself.

Phlorhizin in varying amounts was dissolved in sodium bicarbonate solution, warm Ringer solution, and blood serum, and then added to blood which was incubated for varying periods. Dry phlorhizin also was added to blood in one experiment. The amount of reducing substance lost during the incubation of these mixtures was compared with the loss of reducing substance from an untreated portion of the same blood taken at the same time and incubated for the same period. The results are shown in Table VI.

TABLE VI.

Experiment No.	Phlorhizin.	Solvent.	Glucose before incubation.	Duration of incubation at 38° C.	Glucose after incubation.	Percentile glycolysis.	Glucose in untreated blood after incubation.	Percentile glycolysis in untreated blood.
	mg.		*per cent*	*hrs.*	*per cent*		*per cent*	
III	20	1 cc. 2 per cent sodium bicarbonate	0.102	2	0.077	24.5	0.083	18.6
IV	100	5 cc. 2 per cent sodium bicarbonate	0.152	3	0.103	32.2	0.93	38.8
V	100	5 cc. 2 per cent sodium bicarbonate	0.151	3	0.107	29.1	0.102	32.4
VIII	100	5 cc. 2 per cent sodium bicarbonate	0.144	2⅙	0.095	34.2	0.126	12.5
IX	50	5 cc. Ringer solution	0.098	3	0.162	39.6	0.80	18.3
X	50	5 " " "	0.174	2⅓	0.132	24.7	0.119	31.2
XIII*	60	10 cc. serum	0.152	3	0.185	26.5	0.103	32.2
XV	100	Dry	0.174	5	0.142	18.4	0.120	31.0
XV	50	5 cc. Ringer solution	0.174	5	0.109	37.0	0.120	31.0

* In this experiment deduction was made for the amount of sugar contained in the 10 cc. of serum used to dissolve the phlorhizin. The phlorhizin was partly in solution and partly in suspension.

In six of the nine experiments the difference in the percentile glycolysis between the phlorhizin and the untreated blood was not more than 7 per cent. This difference is too slight to justify an inference that the phlorhizin affected the rate of glycolysis. In Experiment VIII there was apparently an acceleration of the glycolytic process; in Experiment XV apparently a retardation; in Experiment IX instead of a loss during the period of incubation there was an increase of 39 per cent in the amount of reducing substance. I cannot give any satisfactory explanation for this. From these results one cannot conclude that phlorhizin has any effect upon the rate of glycolysis.

CONCLUSIONS.

1. Blood glycolysis *in vitro* during a period of three hours' incubation proceeds at practically the same rate under sterile conditions and when no effort is made to prevent contamination.

2. Fresh thyroid extract, adrenalin, and phlorhizin do not contain any substances which have a constant effect upon the rate of blood glycolysis outside of the body. No evidence of the presence of an antiferment was found.

It is a great pleasure to express my indebtedness to Professor W. G. MacCallum, under whose direction this work was done.

DIET AND TUMOR GROWTH.

By WILLIAM H. WOGLOM, M.D.

(*From Columbia University, George Crocker Special Research Fund, New York.*)

(Received for publication, July 7, 1915.)

One of the most important among many papers dealing with the relations between food and tumor growth was recently published by Van Alstyne and Beebe,[1] who found that a transplantable neoplasm known as the Buffalo rat sarcoma grew better when the hosts were fed on a diet containing carbohydrates (especially lactose) than when they were kept on a non-carbohydrate regimen, provided that these substances were administered continuously for several weeks before inoculation. The enhanced suitability of the lactose-fed animals was sometimes shown by both increased inoculation percentage and more vigorous proliferation of the tumors, at other times by rapid growth alone, the percentage in the latter case being similar to that obtaining for the controls. If these findings were applicable to all propagable neoplasms, the authors point out, it might be possible to rescue a poorly growing tumor, or to obtain large amounts of tissue for experimental purposes; it is evident, also, that the labor and expense incidental to adapting spontaneous new growths for transplantation could be materially curtailed by the simple expedient of keeping on hand a number of lactose-fed animals, a possibility which demanded the fullest investigation.

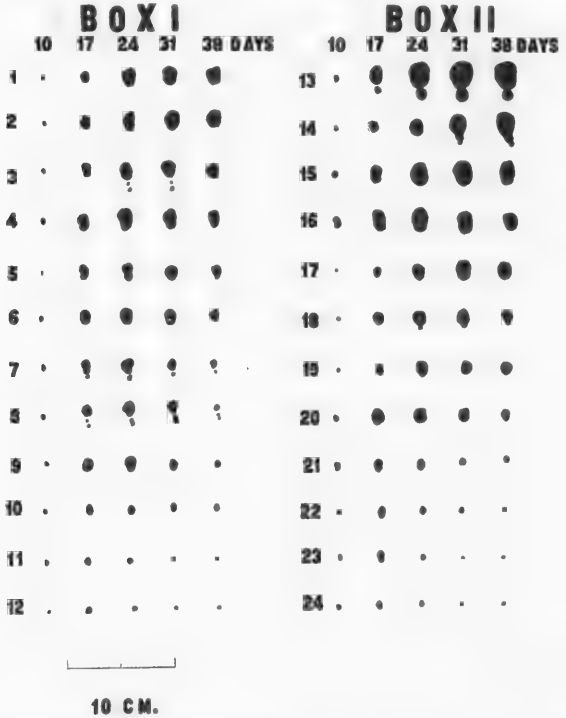
Unfortunately, the various carcinomata employed in the following experiments do not appear to be stimulated by the administration of lactose, although this was given in some instances throughout a period much longer than that regarded by Van Alstyne and Beebe as sufficient; hence, transplantable neoplasms must vary in regard to their sensitiveness toward qualitative differences in diet, as Rous[2]

[1] Van Alstyne, E. V. N., and Beebe, S. P., *Jour. Med. Research*, 1913–14, N. S., xxiv, 217.

[2] Rous, P., *Proc. N. Y. Path. Soc.*, 1914, N. S., xiv, 126; *Jour. Exper. Med.*, 1914, xx, 433.

has shown they differ in respect of their reaction toward quantitative differences, or else the findings of these authors must be referred to the intervention of some uncontrolled factor.

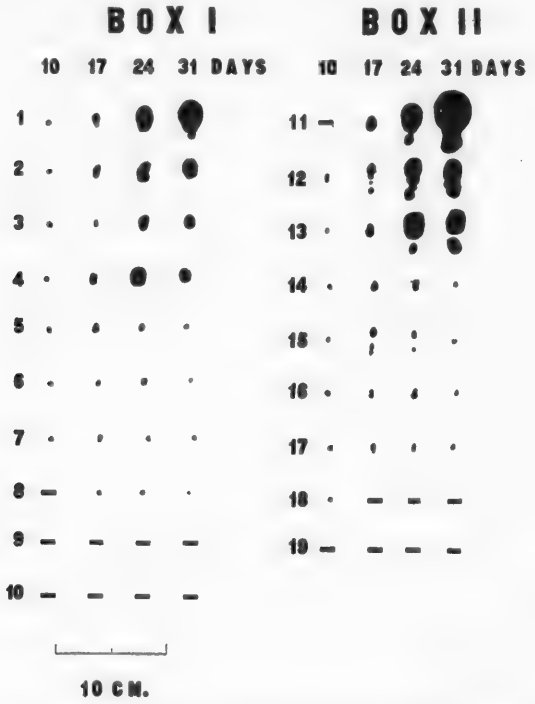
Two objections may be urged against their experiments. These are, first, that their rats may not have been of the same breed, and consequently may not have been equally susceptible to the tumor; and, secondly, that the Buffalo rat sarcoma is one of the most erratic of all the transplantable neoplasms now under investigation. Thus, as Wood and McLean[3] have recently pointed out in a communication from this laboratory, it frequently undergoes spontaneous ab-



TEXT-FIG. 1. Experiment 1. Normal untreated rats bearing the Buffalo rat sarcoma and divided into two groups of twelve each immediately after inoculation.

[3] Wood, F. C., and McLean, E. H., *Proc. Soc. Exper. Biol. and Med.*, 1914–15, xii, 135.

sorption; again, its growth is irregular to the highest degree, as Text-figs. 1, 2, and 3 will demonstrate. These three figures, reproducing pages from the book in which the routine transplantations of this tumor are charted, show how variable is its growth even in normal untreated rats of the same age and breed kept under similar conditions and fed on the same diet, and, at the same time, suggest the employment of a more stable neoplasm for cancer research.



TEXT-FIG. 2. Experiment 2. Normal untreated rats bearing the Buffalo rat sarcoma and divided into two groups immediately after inoculation.

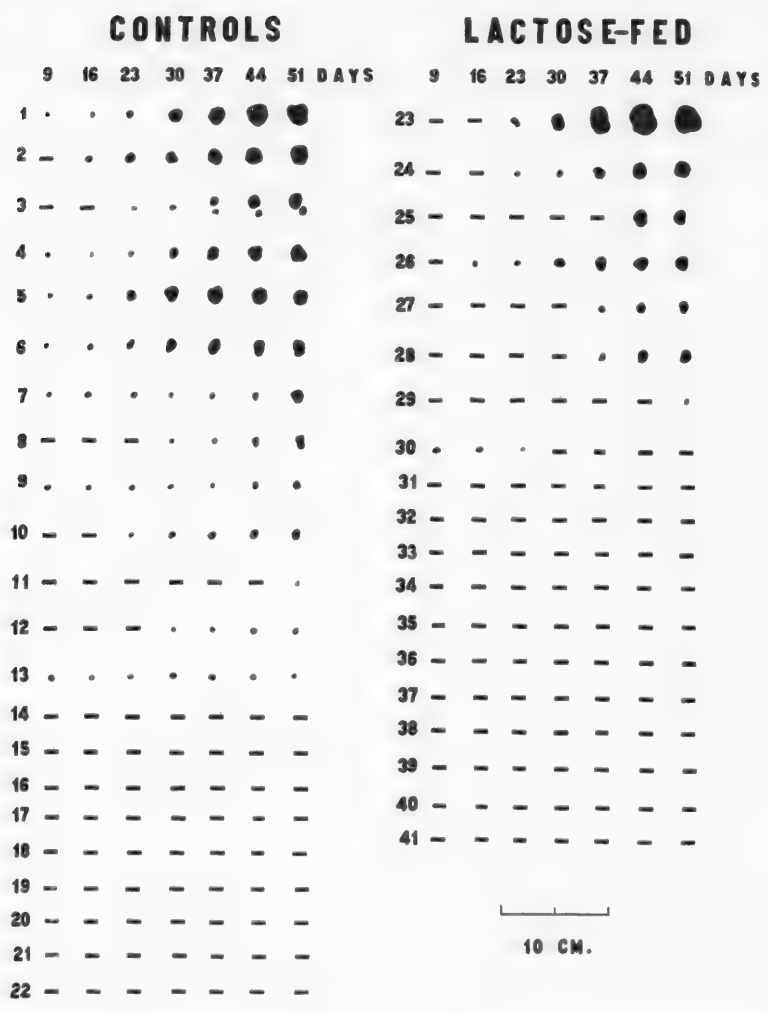
To rule out as far as possible the two variables just cited, the experiments were repeated on mice of the same breed, and with transplantable carcinomata, since these are generally somewhat more uniform than the sarcomata in their behavior. Various growths were employed, possessing this characteristic in common, that their

proliferative capacity was not of the highest; any stimulation, therefore, howsoever slight, would become immediately apparent. Among the mouse carcinomata, Crocker Fund tumors Nos. 15, 46, and 58 have an average inoculation percentage of about 60, grow rather slowly, and sometimes undergo spontaneous absorption, while the

TEXT-FIG. 3. Experiment 3. Normal untreated rats bearing the Buffalo rat sarcoma and divided into two groups immediately after inoculation.

Ehrlich carcinoma (Ehrlich's *"Stamm 33"*) grows in from 70 to 100 per cent of engrafted animals, and does not often retrogress. Two spontaneous mammary carcinomata, Nos. 36 and 46, were also used, and, finally, the Flexner-Jobling rat adenocarcinoma, with an inoculation percentage of from 50 to 100, moderate growth energy, and a rather pronounced tendency to regress.

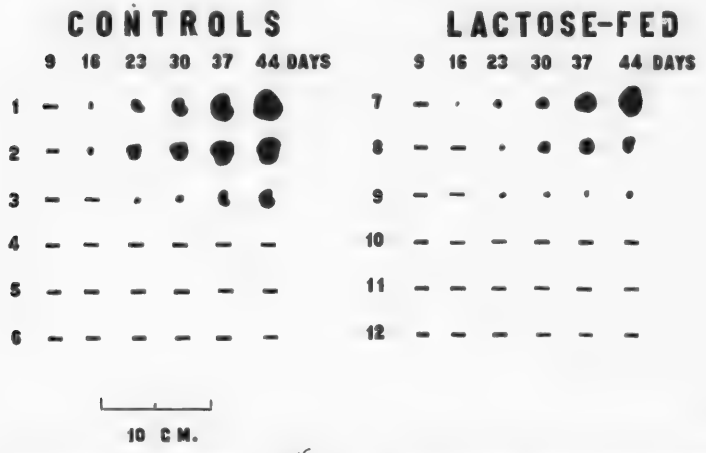
The lactose added in these experiments to the regular laboratory regimen of bread and vegetables was given in lard, sufficient of this



TEXT-FIG. 4. Experiment 4. $\frac{15}{8E}$. Nos. 1 to 22, normal control mice; average weight 15 gm. Nos. 23 to 41, mice fed with lactose for about six months; average weight 22 gm. Both groups inoculated in the right axilla with 0.02 gm. of tumor by the needle method.

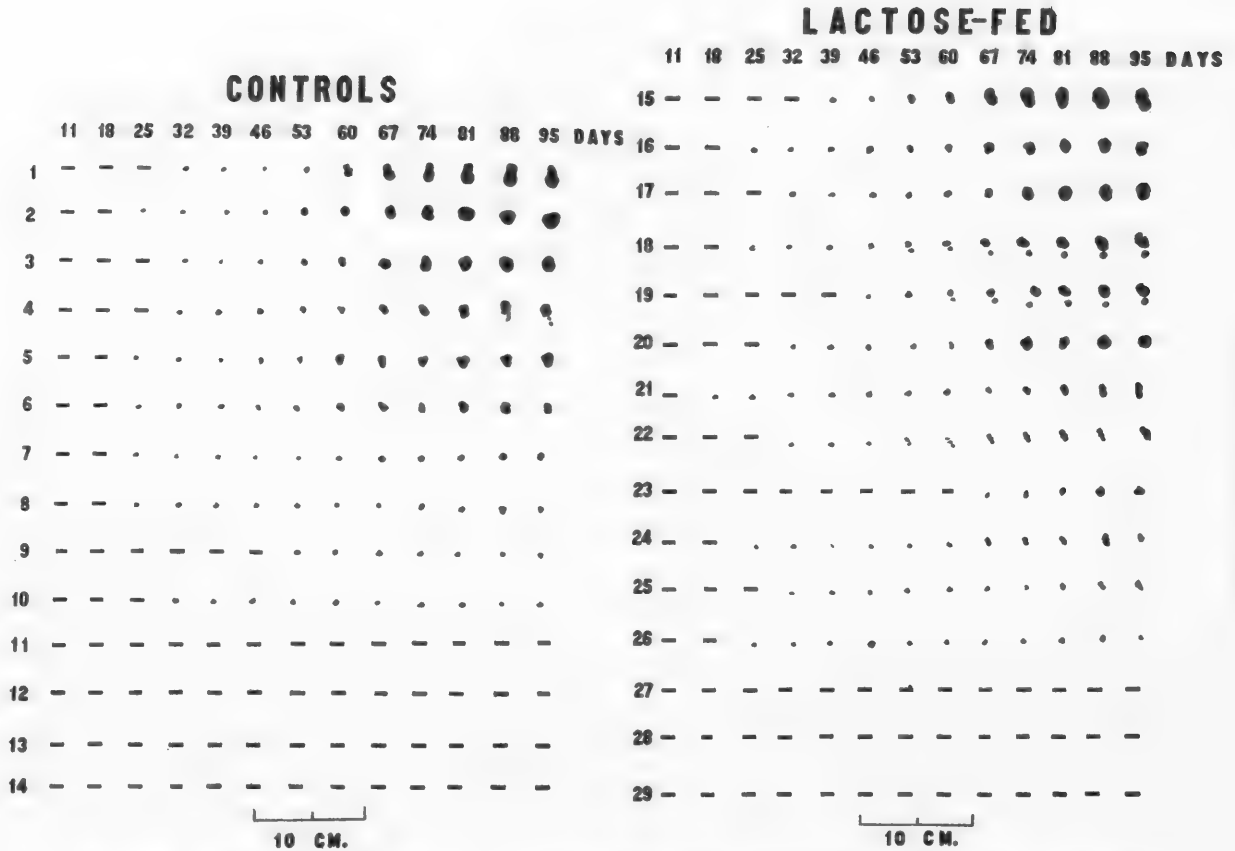
Experiment 4.—(Text-fig. 4.) Only one mouse (No. 23) among the lactose-fed had a larger tumor than the controls, while, totally cancelling this almost negligible difference, the inoculation percentage was a little higher in the control series. If the lactose exerted any effect at all in this group, and in Experiment 6, it was merely to make older mice almost or quite as susceptible as their younger controls, a receptivity far below that described by Van Alstyne and Beebe. The tumor in No. 25 was intra-abdominal, a fact which accounts for its late discovery.

Experiment 5.—(Text-fig. 5.) Here the tumors in the control mice were larger than those in the lactose-fed, the inoculation percentage being identical in the two series.



TEXT-FIG. 5. Experiment 5. $\frac{46}{9D}$. Nos. 1 to 6, normal control mice; average weight 27 gm. Nos. 7 to 12, mice fed with lactose for about six months; average weight 25 gm. Both groups inoculated in the right axilla with 0.02 gm. of tumor by the needle method.

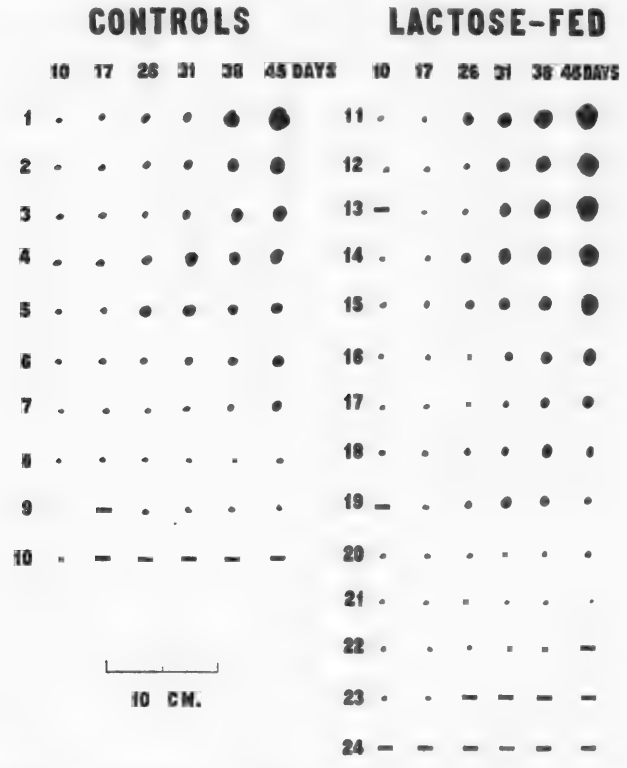
mixture being put into the cages to have some still remaining at the next feeding, and it was continued in each case until the termination of the experiment; the controls received only bread and vegetables. Save for this difference in food, the lactose-fed animals and their controls were kept under identical conditions throughout. Ingestion of the lactose was proved, for the rats at least, by examination of the stomach contents (Experiment 12), those of the group fed with this substance reducing Fehling's solution in every case, a reaction which did not take place in any of the controls. Except in Experi-



TEXT-FIG. 6. Experiment 6. $\frac{58}{3G}$. Nos. 1 to 14, normal control mice; average weight 18 gm. Nos. 15 to 29, mice fed with lactose for about six months; average weight 23 gm. Both groups inoculated in the right axilla with 0.02 gm. of tumor by the needle method.

Experiment 6.—(Text-fig. 6.) Between the two groups there was but slight difference, either in the rate at which the tumor had grown or in the inoculation percentage.

Experiment 7.—(Text-fig. 7.) In this experiment the tumors in the lactose-fed were a little larger than those in the controls, but the inoculation percentage was about the same.



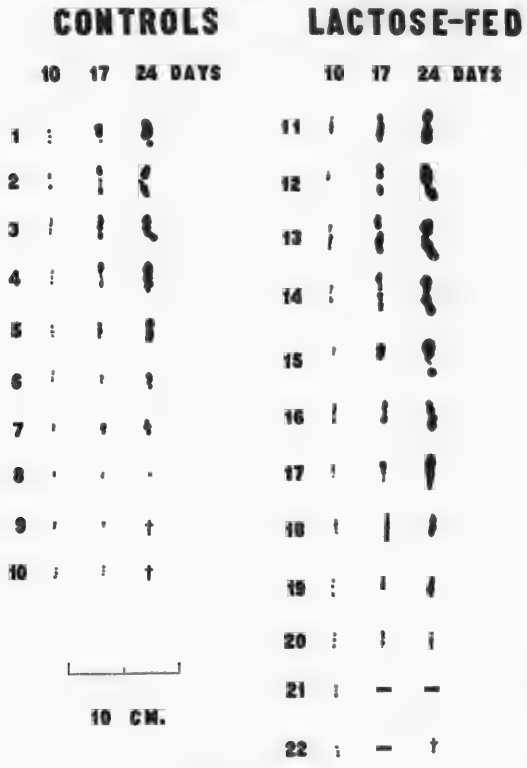
TEXT-FIG. 7. Experiment 7. $\frac{EC}{99B}$. Nos. 1 to 10, normal control mice. Nos. 11 to 24, mice fed with lactose for about two months. Both groups inoculated in the right axilla with 0.01 gm. of tumor by the needle method. On account of an oversight, the mice of this experiment were not weighed.

ments 4, 6, and 12, the weights of the animals were approximately equal. In 4 and 6, however, the controls set aside at the beginning of the experiment had to be replaced by younger animals of the same breed, as they had all died when the time came for inoculation. For

TEXT-FIG. 8. Experiment 8. $\frac{EC}{98A}$. Nos. 1 to 18, normal control mice; average weight 15 gm. Nos. 19 to 36, mice fed with lactose for about one month. Average weight 17 gm. Both groups inoculated in the right axilla with 0.02 cc. of a tumor emulsion by the syringe method.

Experiment 8.—(Text-fig. 8.) The tumors were somewhat larger in the control mice, and the inoculation percentage was practically similar in the two groups.

Experiment 9.—(Text-fig. 9.) Here the tumors were a trifle larger in the lactose-fed series, though the inoculation percentages were approximately equal.

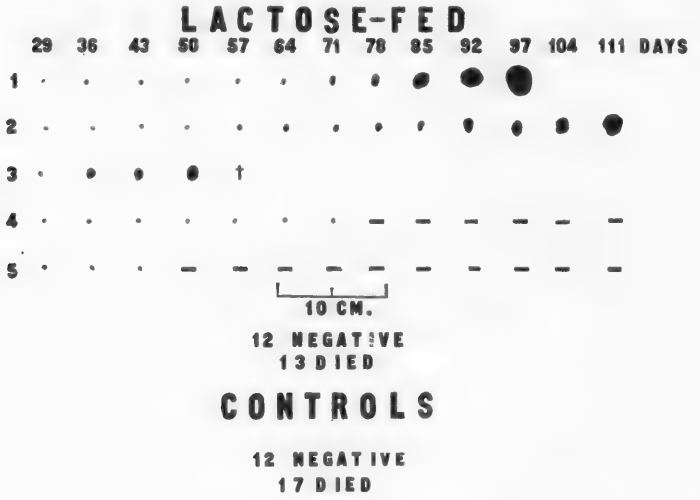


TEXT-FIG. 9. Experiment 9. $\frac{EC}{4B}$. Nos. 1 to 10, normal control mice; average weight 16 gm. Nos. 11 to 22, mice fed with lactose for about six weeks; average weight 17 gm. Both groups inoculated in the right axilla with 0.02 cc. of a tumor emulsion by the syringe method.

Experiment 12 it was not possible to get rats of exactly the same size; here, however, the lactose-fed group was the younger.

Summing up, it appears that in a total of nine experiments comprising 123 lactose-fed animals and 109 controls, the tumors were

Experiment 10.—(Text-fig. 10.) This is the only one of the entire series which would even suggest that a diet containing lactose may render an animal more suitable for tumor growth. Of seventeen lactose-fed mice which lived long enough to appear in the final reckoning, three had fairly large neoplasms, while not one of the twelve controls developed a tumor.



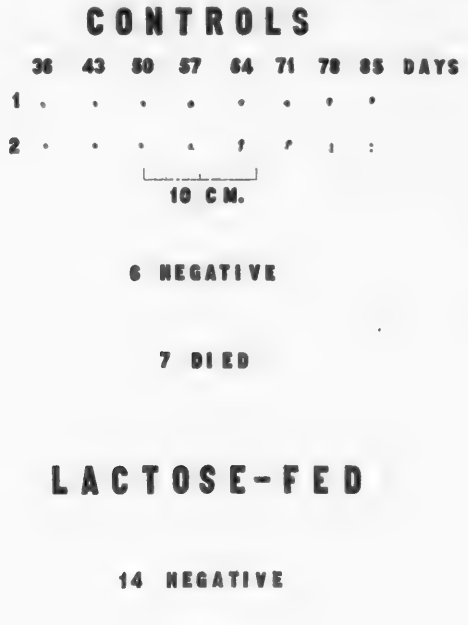
TEXT-FIG. 10. Experiment 10. $\frac{46}{1}$. Nos. 1 to 5, mice fed with lactose for about six weeks; average weight 16 gm. The average weight of the control mice was also 16 gm. Both groups inoculated in the right axilla with 0.01 gm. of tumor by the needle method.

slightly larger or the inoculation percentage a trifle higher in the lactose-fed mice in three, in the control mice in three, and in three no difference could be discerned between the two groups. Such an even distribution makes it almost certain that the addition of lactose to the diet did not in the slightest affect the growth of the tumors employed, and intimates, though of course it does not prove, that the findings published by Van Alstyne and Beebe may have been the outcome of chance.

Other substances beside lactose have been brought recently into connection with tumor growth. Robertson and Burnett[4] have as-

[4] Robertson, T. B., and Burnett, T. C., *Proc. Soc. Exper. Biol. and Med.*, 1912–13, x, 59, 140. Burnett, T. C., *ibid.*, 1913–14, xi, 42; 1914–15, xii, 33. Robertson and Burnett, *Jour. Exper. Med.*, 1913, xvii, 344.

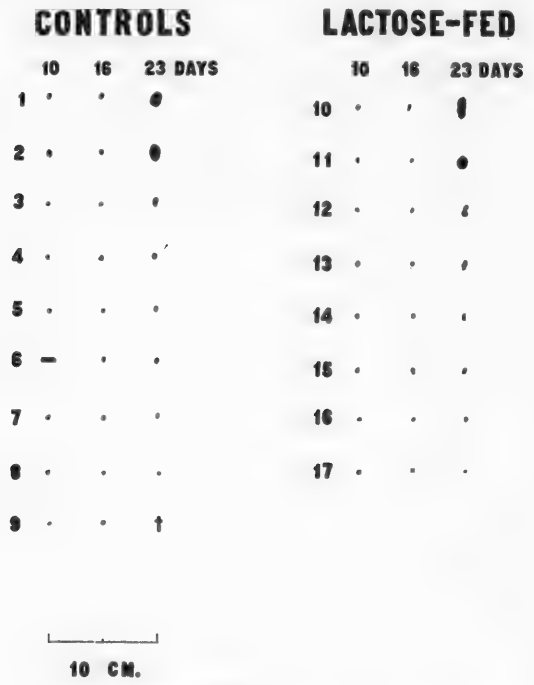
Experiment 11.—(Text-fig. 11.) This experiment reverses the findings of the previous experiment, since the only tumors which were found occurred in the controls. Both, however, were much smaller than the three mentioned in Experiment 10.



TEXT-FIG. 11. Experiment 11. $\frac{36}{1}$. Nos. 1 and 2, normal control mice; average weight 22 gm. The lactose mice, which averaged 24 gm. in weight, had been fed with lactose for about six weeks. Both groups inoculated in the right axilla with 0.02 gm. of tumor by the needle method.

serted, for example, that cholesterol acts as a stimulus when injected into the tumor or even when introduced subcutaneously at a remote point. Now, while this may or may not be true, the authors have fallen into an error which Van Alstyne and Beebe carefully avoided, the only too common error of confusing tumor growth with tumor genesis. In other words, they have extended their findings to explain the constantly increasing tendency toward neoplasia which is associated with advancing age, and have ascribed it to an accumula-

Experiment 12.—(Text-fig. 12.) There is no difference between the two groups, either as regards the size of the tumors, or the inoculation percentage, notwithstanding the fact that the lactose-fed rats were younger than the controls.



TEXT-FIG. 12. Experiment 12. $\frac{FRC}{3E}$. Nos. 1 to 9, normal control rats; average weight 65 gm. Nos. 10 to 17, rats fed with lactose for about six weeks; average weight 52 gm. Both groups inoculated in the right axilla with 0.02 gm. of tumor by the needle method.

tion of cholesterol within the organism. Such a broad assumption is scarcely warranted by any of the data which it has been possible so far to accumulate; indeed, all the evidence points to an opposite conception—that the conditions responsible for the origin of a tumor differ widely from those necessary for its continued growth, once it has developed. This hypothesis, accepted by both Bashford and Ehrlich, rests on the complementary observations that spontaneous

tumors sometimes arise in mice known to be refractory to transplantable growths, and that grafts succeed much more readily in young than in old animals, although it is in the latter that spontaneous neoplasms originate in the great majority of instances.

SUMMARY.

Lactose does not increase the receptivity of mice and rats for the transplantable carcinomata of their species.

OBSERVATIONS ON ANTITYPHOID VACCINATION.[1]

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In this paper, some evidence, experimental or clinical, is presented on the following subjects: (1) The infectious power of a living, sensitized vaccine; (2) the absence of immunity in vaccinated rabbits to direct gall bladder infections; (3) the relative pathogenicity, virulence, and toxicity of the strain used in the Army vaccine; (4) local vaccine reactions after typhoid fever and after immunization; and (5) skin reactions as an index of immunity.

The Infectious Power of a Living Sensitized Vaccine.

I have recently reported (1) some work on this subject in which it was shown that Metchnikoff and Besredka's sensitized living vaccine does not produce gall bladder lesions in the rabbit after subcutaneous and intravenous injections, and the inference was drawn that this vaccine could safely be used subcutaneously in man. The first transplant of the vaccine, however, produced a gall bladder lesion in the rabbit after intravenous injection, and it was inferred that this vaccine would produce the disease in man if accidentally taken by mouth. Its general use, especially in the military service, was, therefore, concluded to be dangerous.

A little further work has been done. On Oct. 28, 1914, three rabbits were inoculated directly into the gall bladder with 1 cc. of Metchnikoff and Besredka's vaccine. The vaccine was then more than one year old, but still gave a growth of typhoid bacilli in pure culture. On Nov. 10 the stools of the three animals were examined by Endo plates and Russell's double sugar media method, and typhoid bacilli were easily recovered from each of the three animals. On autopsy

[1] Read at the Annual Meeting of the American Society of Tropical Medicine, San Francisco, June 15, 1915.

a purulent cholecystitis was found, and pure cultures of the typhoid bacillus were cultivated from the pus in all three animals.

This experiment confirms the former one done with the first transplant of the vaccine because the bile in this instance was simply a good medium for multiplication. Apparently, therefore, this strain is infectious, unless it is exposed to both amboceptor and complement. If it is not treated with amboceptor it is infectious, as when introduced into the blood stream; if it is sensitized but not exposed to complement it is also infectious, as when introduced directly into the gall bladder. Since neither amboceptor nor complement are present in the alimentary canal, any food or water accidentally contaminated with this vaccine would be apt to spread the disease among non-immunes. From this point of view, this vaccine is simply a pure culture of pathogenic typhoid bacilli and should be treated accordingly. The toxicity of a living sensitized vaccine for rabbits and guinea pigs is considerably less than that of an unsensitized vaccine, as has been shown by Besredka (2) and by Cecil (3); but its infectious power is unimpaired except when complement is present.

Absence of Immunity in Vaccinated Rabbits to Direct Gall Bladder Infections.

The claim for the superiority of a living antityphoid vaccine rests on analogy and on experimental work done with the higher monkeys, which cannot be easily repeated on account of the difficulty of obtaining the animals. The rabbit is not a suitable animal for testing immunity following vaccination, in my experience, at least, because regular infections of the gall bladder cannot be secured by intravenous injections among controls, and because direct gall bladder injections result in lesions in spite of any method of immunization. This last point is illustrated by the following experiment.

Three rabbits were immunized with Besredka's vaccine as follows:

1.....Dec. 24, 1914, 0.5 cc. subcutaneously.
2..... " 31, " 1.0 " intravenously.
3.....Jan. 7, 1915, 1.0 " "

On Jan. 24 the gall bladders were exposed, and after withdrawing about 0.5 cc. for culture, 0.5 cc. of the living vaccine was injected. Preliminary cultures of

the bile were sterile. On Feb. 8, 12, and 22 the animals were autopsied and all showed a marked purulent cholecystitis, with pure cultures of typhoid bacilli.

Hence in this experiment no immunity could be demonstrated with a living vaccine when the test injection was given directly into the gall bladder. Similar results have been obtained by others using other forms of vaccine, and it is evident that the rabbit cannot be used for testing immunity experimentally under these conditions.

Living cultures, unsensitized but heated to 50° C. for one hour, have been used without bad results by Castellani (4). No personal work has been done with this form of vaccine, but it seems likely that this vaccine would also be infectious if accidentally taken by mouth, as bacilli are readily cultivated from it on agar. The attenuation by heat apparently makes it safe for subcutaneous injections and probably small quantities of even a virulent culture could be safely injected subcutaneously. But in view of the excellent results obtained with an absolutely non-infectious, killed, and cresolized vaccine, and in view of the evident infectiousness by mouth of a sensitized living vaccine, and in view of the added danger of accidental contamination of a living vaccine itself such as occurred in the Philippines and in India with living cholera and plague vaccines, the field of usefulness of a living antityphoid vaccine does not seem to be very wide.

The Relative Pathogenicity, Virulence, and Toxicity of the Army (Rawling's) Strain.

The most convincing clinical evidence of the protective value of antityphoid immunization has been furnished by the recent experiences of the English and the American armies. The same single strain has been used in the preparation of the vaccine in both services, and substantial protection has been secured in spite of exposures to typhoid in all parts of the world. In our service, the latest statistics (5) show no lowering of our previous remarkable records, but failure in immunization has been reported in several civil communities (6). In view of the results, some interest attaches to the Army strain and some observations have been made on its relative pathogenicity, virulence, and toxicity, as compared with nine other strains. The strain was originally obtained from the spleen of a

soldier who died of typhoid in England in 1900. It was selected originally by Leishman for experimental use in preparing vaccines, not on account of its low toxicity or superior immunizing properties, but because it gave a remarkably even emulsion when washed off agar with salt solution (7).

Culturally no peculiarities have been noted as far as I am aware.

Pathogenicity.—The strain is still pathogenic, as may be seen in the following experiments.

On Oct. 8, 1914, three rabbits were inoculated directly into the gall bladder with 1/20 of a fresh agar slant growth; a month later, autopsy showed a definite cholecystitis with pure cultures of the typhoid bacillus in two out of three animals. The strain is therefore still definitely pathogenic.

Virulence.—As gall bladder inoculations with many other strains give 100 per cent of infections, the result just stated suggests that the strain is somewhat avirulent. Further evidence on this point is brought out by intravenous injections in the rabbit. Six animals were inoculated intravenously with one-half the fatal dose of living bacilli, and after a month no gall bladder lesions were found in any of them. With more invasive strains a certain percentage of infection is usually secured in this way, about 30 per cent in my experience.

TABLE I.

Strain No.	Age.	1/10	1/7	1/5	1/4	1/2	1
1	1 week	—	×*	×	×		
2	1 month					×	×
3	" "	—	×	×	×		
4	2 months	—	—	×	×		
5	7 "	—	—	×			
6	8 years	—	—	×	×		
7	" "	—	—	—	—		
8	?	—	—	—	×		
9	14 years	—	—	—	—		
Army	" "	—	—	—	—	—	—

* In all the tables × indicates death in 24 hours.

Guinea pigs are not quite as suitable as rabbits for the determination of virulence, because it is not so easy to verify the multiplication of the bacilli, but guinea pigs have usually been used in this work, and the results here also point to the low virulence of this strain. In Table I the comparative results are given of intraperito-

neal injections in guinea pigs of the Army strain and nine other strains. The figures indicate fractions of a standard agar slant growth.

It is seen that the more recently isolated strains are more virulent in general, but some of the older strains are still virulent. The Army strain fails to kill after injection of a whole culture. In one series the growth from two and one-half agar slants was required to kill a guinea pig.

Toxicity.—Virulence is often spoken of as identical with toxicity in work of this sort, but in my experience real virulence or invasiveness seems to have no definite relation to toxicity. The strains which, when living, are more virulent for rabbits and guinea pigs have, when killed, no more toxic effects on rabbits and guinea pigs than the strain in question. No consistent toxic action can be obtained with the more virulent strains in smaller quantities than with the Army strain. This subject is of some clinical importance, as local and general reactions seem to depend directly on toxicity rather than on virulence.

The toxicity of a vaccine can be experimentally determined by the results of intravenous injections in rabbits. For example, the effect of a colon vaccine can be cited. As is well known clinically, a colon vaccine gives severe local and general reactions unless administered in small quantities, such as tens of millons rather than in hundreds of millions, as in the case of typhoid vaccines. And a colon vaccine will kill a rabbit in a much smaller dose than is required in the case of a typhoid vaccine. A number of observations have been made on the toxicity of the Army vaccine for rabbits, after intravenous injection. The results obtained by using different lots kept for different times are shown in Table II. The doses are in cc. per kilo of body weight.

TABLE II.

Period kept.	3 cc.	4 cc.	5 cc.	Period kept.	3 cc.	4 cc.	5 cc.
1 month	×(×)*	×		8 months	(×)—	—	
2 months	(×)			9 "			—
3 "	—	×		10 "	—	—	—
5 "	(×)			11 "			—
6 "	(×)—	—	—	12 "	—	—	—
7 "			—				

*In the tables (×) indicates death later from cachexia.

A certain amount of allowance must, of course, be made for differences in individual rabbits, but this result shows that the vaccine is distinctly toxic, especially during the first three months.

As was said before, more virulent strains tested in the same way have not proved any more toxic. The results of the use on rabbits of fresh vaccines made from three most virulent strains are as follows (Table III).

TABLE III.

Strain No.	3 cc.	4 cc.	5 cc.
1	—	—	—
2	—	—	×
3	×	—	—

As can be seen, no consistent increase in toxic action of more virulent strains can be demonstrated.

Period of Maximum Toxicity.—One lot of the regular vaccine was tested every month for six months; the vaccine was kept on ice except during shipment across the country. The results are shown in Table IV.

TABLE IV.

Period kept.	3 cc.	4 cc.	5 cc.	Period kept.	3 cc.	4 cc.	5 cc.
1 week	—	—	—	4 months	—	—	—
1 month	—	—	—	5 "		—	—
2 months	—	—	—	6 "		—	—
3 "	(×)	×	×				

This result seems to indicate that the toxicity increases with age and is at its maximum at three months. At present the bulk of the Army vaccine is used only in the first four months after manufacture. Apparently there is a gradual disintegration of the bacilli with greatest liberation of endotoxins at three months.

Increased Toxicity by Killing Bacilli with Tricresol.—The method used in preparing the Army vaccine consists of killing at 53° to 54° C. for one hour and then adding 0.25 tricresol as a precautionary antiseptic. The heating kills nearly all the bacilli, although a few colonies usually develop on plating 1 cc. before adding tricresol. It has been proposed to kill the bacilli directly by the tricresol; this procedure increases the toxicity of the vaccine as may be seen from Table V.

TABLE V.

Method of preparation.	Dose per kilo intravenously.		
	2 cc.	3 cc.	5 cc.
1. Fresh vaccine, heated at 53°–54° C. for 1 hr.	—	—	
2. Fresh vaccine, heated and tricresolized	—	—	
3. Fresh vaccine, tricresolized but not heated	×	×	
Control: tricresolized sodium chloride		—	—

Both rabbits given the tricresolized unheated vaccine died in twenty-four hours, while the others were not seriously affected. When the bacilli are treated directly with tricresol, the endotoxins are apparently liberated more rapidly than when the bacilli are first killed with heat.

DISCUSSION.

From the results given above I believe that a rationale of the action of the Army vaccine can be formulated as based on its toxicity. The strain used, while avirulent, is pathogenic and distinctly toxic. In preparing the vaccine, no effort is made to reduce its toxicity, but at the same time procedures which exaggerate the toxic action are avoided. The doses given are the maximum ones which can be borne without serious disturbance by the majority of individuals. The doses are spaced at seven or ten days in order to secure a response to the gradually liberated endotoxins. Finally, the vaccine is not used after four months, when its toxicity is on the wane. I do not claim that the toxic action of the vaccine has been the leading consideration in all the steps of its development, but I do hold that the net result conforms to the theory that the efficiency of the Army vaccine depends on its toxicity.

Various workers have attempted to devise atoxic vaccines by sensitization, fractionation, etc. From the point of view outlined above, such efforts belong to the "something for nothing" class. The most reasonable theory of the action of the vaccine seems to me to be that of Pfeiffer and Bessau (8) who hold that the toxic and immunizing fractions are identical, and that efforts to separate them are wrong in principle. Fortunately, from this point of view, it is not easy to prepare a really non-toxic antityphoid vaccine. The toxic action may be masked or slight, but it is demonstrable in most vaccines called atoxic. In the opinion of those who have been

most responsible for the introduction of this method of protection, something must be paid for immunity in terms of local or general reactions (Leishman (9)).

The theory of endotoxins received a set-back during the recent split products and ferment period, but as some of this latter work has still more recently been shaken to its foundations by the work of Jobling (10), Bronfenbrenner (11), and others, the endotoxin theory seems to still be the best working hypothesis for immunization and for keeping qualities of the vaccine.

Local Vaccine Reactions after Typhoid Fever and after Artificial Immunization.

A careful study of a very large number of temperature reactions following vaccination against typhoid has been made by Major Russell (12), and the figures show a very small percentage of reactions of 100° F. or over. The temperature reaction was selected as the most objective evidence which could be accurately obtained. Some misapprehension has apparently been created by these figures on account of a failure to recognize what they really are, namely, temperature reactions, which are not necessarily an index of a patient's general feeling nor of the local reaction. From what has been said about the toxicity of the vaccine it is only natural to expect some local and general reaction, and in fact this price is usually paid for protection, although the temperature is not much affected.

A similar study of temperature reactions as a result of a vaccination in those who have had typhoid fever, or have been previously artificially immunized, has also been made by Major Russell, and no notable changes in the percentages have been forthcoming. On the other hand the local reactions under the skin seem to be more marked in some cases after typhoid fever and after vaccination. I have no exact statistics on this point but the consensus of opinion is that the local reactions may be more severe than on primary vaccination. In the more marked cases the arm may become intensely red and swollen from the elbow nearly to the shoulder. I have recently seen a soldier who volunteered for a course of injections whenever the subject was mentioned, and who had taken ten injections in the last five years. Through an oversight he was

given another injection, and as a result he had to carry his arm in a sling for several days. His temperature remained normal. On the other hand, a number of men, who have had typhoid or two courses of three injections each, on revaccination had very little local reaction.

The explanation of these reactions involves complex questions. They may be regarded as evidences either of true immunity, or of sensitization, or of endo-intoxication, according to one's conceptions of these complicated subjects. The point I wish to emphasize is that the reactions do not occur regularly either after typhoid or after vaccination. Exact percentages cannot be given but my impression is that they occur in about 50 per cent of cases.

Skin Reactions as an Index of Immunity.

The subject of typhoid skin reactions has recently been revived by Gay (13) who has proposed the use of typhoidin as an index of immunity. The first preparation advocated was one made like tuberculin, by growing the Dorset strain in glycerine broth for five days, concentrating to one-tenth of its volume, and using as a dermal reaction. My experience with this product was disappointing, as several cases who had had proved cases of typhoid failed to react at all, and such reactions as were obtained among the vaccinated were very faint. This failure has been attributed to a rapid deterioration of the typhoidin, and more recently a powder has been advocated which is prepared by precipitating the original solution with twenty volumes of alcohol, washing the precipitate with alcohol and ether, and drying and grinding.

Professor Gay has kindly furnished me with some of the powdered typhoidin, and I have made seventy-five tests on sixty-seven individuals. The reactions with the powder have been much more satisfactory than those with the liquid form. Positive reactions were obtained in a fairly large proportion of persons who had either had typhoid or been vaccinated. At the same time, it seems to me that the results are open to a different interpretation from the one given by Gay. From my experience with the test I regard a positive reaction as an evidence of typhoid proteid sensitization which does not seem to be synonymous with true disease immunity. For

example, typhoid proteid immunity is apparently less lasting than true immunity to the disease and less specific, as will be shown below.

The immunity conferred by an attack of typhoid is the starting point of immunization by vaccines, and the test should give a high percentage of positive reactions among those who have had the disease. Gay states that the test was positive in his series in 97 per cent of cases. The only cases of previous typhoid available for personal observation have been sixteen, thirteen of whom had also been vaccinated three years previously. Twelve cases, or 75 per cent, gave positive reactions and this must be a maximum figure on account of the additional vaccination. Seven of the twelve positive cases gave marked reactions.

As an attack of typhoid fever is generally believed to give immunity in over 90 per cent of cases, my figure of 75 per cent, even in those artificially immunized in addition to their fever, is lower than it should be in a test for immunity, while it can be better harmonized with the interpretation suggested above. Of course, the question of diagnosis of the previous fever comes up and this was determined to my satisfaction by the histories given. The subject of the reading of the test is also a factor. The interpretation of slight reactions is difficult at times, as the culture media control occasionally gives slight reactions, but an effort was made to read each individual case as fairly as possible.

The test was then tried in fifty cases who never had typhoid and who had been immunized within four years; 64 per cent gave positive reactions, as may be seen in Table VI.

TABLE VI.

Reactions.	1st year.	2d year.	3d year.	4th year.	Total.
Positive	19	5	5	3	32
Negative	4	4	7	3	18
Per cent positive	83	56	42	50	64

It will be seen that in the first year after vaccination there was a high percentage of positives, but after this year the percentage was lower and about the same up to the fourth year. Eighteen cases were tested one month after completion of a course of three injec-

tions and only fourteen, or 78 per cent, gave positive reactions, of which 60 per cent were strong. Among those immunized from one to four years ago there were no strong reactions. If the typhoidin test is an index of immunity according to these results nearly one-fourth of those vaccinated a month previously are not protected and over one-third of those vaccinated up to four years are not protected. But our experience in the Army has not borne out this view, as our protection is very much greater for the periods mentioned.

The most important reason why the test is not an index of immunity, in my opinion, is the occurrence of reactions with a paratyphoid A control (Table VII). Twenty-four cases have been tested; five gave a history of typhoid and nineteen had been vaccinated against typhoid. Sixteen, or 66 per cent, gave reactions, eight, or 33 per cent, of which were strong.

TABLE VII.

Reactions.	Previous typhoid.	Vaccinated against typhoid.	Total.	Per cent.
Para A control				
Positive	3	13	16	66.6
Negative	2	6	8	
Typhoidin				
Positive	2	16	18	75
Negative	3	3	6	

Apparently we are dealing here with group reactions. The percentage of positives with the paratyphoidin was not quite as high as that of the typhoidin reactions, neither were they as strong, but the close parallelism is striking. If this skin reaction is an index of immunity, there should be considerable immunity following antityphoid vaccination against paratyphoid infections. But one of the clear cut results of typhoid immunization is that there is no group immunity to paratyphoid infections and where these diseases are prevalent a combined vaccine has been urged by Castellani and others. Both in the English service and in our own the occurrence of paratyphoid among those immunized against typhoid has been emphasized and in the present war Dreyer (14) and others have just reported that paratyphoid is of the same frequency among those vaccinated against typhoid as among those not vaccinated.

The nature of the proposed typhoidin test is still an open ques-

tion. For other clinical skin tests we have some approximate explanation. The tuberculin and luetin reactions are supposed to depend in some way on the continued presence in the body of the living virus of the diseases; the Schick test apparently depends on an antitoxin; but in the typhoidin test we have to deal, not with an antitoxin or with a living virus, but with the complex effect of the previous presence of typhoid bacilli in the body. The same questions are raised as in the case of the local subcutaneous reaction already mentioned. The reaction may well be an evidence of proteid immunity without being an evidence of true immunity to the disease.

From my experience, the following reasons may be advanced for a different interpretation of the typhoidin skin reaction:

1. The test was negative in 25 per cent of cases who have had typhoid and most of whom had also been vaccinated, while an attack of typhoid fever is supposed to give permanent immunity in over 90 per cent of cases.

2. It was negative in 36 per cent of those who have been vaccinated within four years, while experience has shown that vaccination protects in much more than 64 per cent of cases.

3. Paratyphoid A controls reacted in nearly as high a percentage (66) as typhoidin (75), while clinical experience is unanimous that there is no immunity to paratyphoid infections after typhoid immunization.

Personally it seems to me that a positive typhoidin reaction is of considerable theoretical interest and indicates that the individual reacting has had typhoid fever or possibly paratyphoid or has been injected with typhoid bacilli. A negative reaction does not have the same negative value and is not so conclusive.

SUMMARY.

1. Metchnikoff and Besredka's living sensitized vaccine produces a typhoid cholecystitis when injected directly into the gall bladder of rabbits. It is therefore infectious.

2. Rabbits cannot be successfully immunized with this vaccine against direct gall bladder infections. Accordingly, rabbits cannot be used to test immunity in this way.

3. The strain used in the Army vaccine is pathogenic, relatively avirulent, and distinctly toxic. Its efficacy is believed to depend on its toxicity.

4. Vaccinations in those who have had typhoid and revaccinations produce more severe local reactions than original vaccinations in some instances.

5. The typhoidin skin test is not believed to be an index of true immunity, but rather an indication of typhoid proteid sensitization, which is not so complete, so permanent, or so specific as true immunity.

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14. Dreyer, G., Walker, W. A., and Gibson, A. G., *Lancet*, 1915, i, 324.

THE RELATIONSHIP OF CHRONIC PROTEIN INTOXICATION IN ANIMALS TO ANAPHYLAXIS.[1]

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The experiments which furnish the subject of this paper represent a continuation of some observations made upon the effect of repeated injections of foreign protein in animals sensitized to these specific proteins.[2] Under these conditions areas of degeneration with extensive inflammatory reaction are produced in the myocardium, the liver, and the kidneys. In different species of animals the effects vary somewhat, since the kidney is involved in the dog, cat, rabbit, and guinea pig, the liver in the rabbit and cat,[3] and the myocardium[4] in the rabbit and guinea pig. A detailed description of these changes has already been published, and it is, therefore, necessary only to state that they consist in focal areas of degeneration, infiltrated with small round cells, which in the heart are scattered throughout the myocardium of both ventricles, in the liver are usually situated about the portal spaces, and in the kidney are seen most often through the midzone, whence they extend into the cortex. In their most advanced stages these alterations produce the picture of an extensive myocarditis, a periportal cirrhosis of the liver, or a wide-spread subacute nephritis with involvement, at times, of the glomeruli.

At first it was thought that sensitization to some foreign protein, such as horse serum or egg-white, was necessary before subsequent injections of the same protein could bring about the development of such changes, but further study showed that similar, though

[1] Read before the Association of American Physicians, Washington, May 10, 1915.

[2] Longcope, W. T., *Jour. Exper. Med.*, 1913, xviii, 678.

[3] Longcope, *Tr. Assn. Am. Phys.*, 1913, xxviii, 497.

[4] Longcope, *Arch. Int. Med.*, 1915, xv, 1079.

less marked, alterations might occur in animals receiving but a single large dose of horse serum or egg-white. The question, therefore, arose as to whether the effects produced were the result of introducing repeatedly into the bodies of these animals small quantities of a substance which was in itself primarily toxic for the cells of the kidneys, liver, and myocardium, or whether the horse serum and egg albumen became injurious only after the animals were sensitized to these substances. In the latter case the subacute inflammatory reactions might readily be dependent upon the repeated anaphylactic shocks to which the animals were subjected.

Though a number of observers interested principally in the fact that egg-white, when introduced parenterally into animals, is eliminated as such in the urine, have studied casually the kidneys of animals soon after an injection of egg-white, very few have described definite alterations in the kidneys. Adams[5] and Maschke[6] state that the kidneys of rabbits after single injections of egg-white are normal. Sollmann and Brown[7] observed under the same conditions occasional swelling and degeneration of the tubular epithelium. Chiray[8] could not discover any changes in the kidneys of rabbits after a single intravenous, intraperitoneal, or subcutaneous injection of egg albumen, but noted after repeated injections, which were limited to a few animals, degeneration of the tubular epithelium with cellular reaction and scar formation that led to chronic nephritis.

The following experiments were undertaken with the view of determining, if possible, whether the toxic action of horse serum and egg-white was a primary property of these proteins or developed only when the animals were sensitive to such foreign proteins. The latter view would express the idea which has long been held by Vaughan.[9]

It was thought that if the protein itself were toxic, it might be possible to demonstrate this toxicity by injecting repeatedly at weekly intervals proteins always of a different type, so that the animal might receive large quantities of foreign protein but never the same protein twice, thereby avoiding anaphylactic shock.

The following experiment was, therefore, performed:

[5] Adams, B., Inaugural Dissertation, Leipzig, 1880.

[6] Hirsch, C., and Maschke, W., *Berl. klin. Wchnschr.*, 1912, xlix, 145.

[7] Sollmann, T., and Brown, E. D., *Jour. Exper. Med.*, 1902, vi, 207.

[8] Chiray, M. M., Thèse de Paris, 1906.

[9] Vaughan, V. C., Vaughan, V. C., Jr., and Vaughan, J. W., Protein Split Products in Relation to Immunity and Disease, Philadelphia and New York, 1913.

Four lots of rabbits were selected from a single stock. Lot A, consisting of 11 rabbits, was used as control. Lot B, consisting of 11 rabbits, was sensitized to horse serum or egg-white by intraperitoneal inoculation of 2 to 4 cc. of these proteins on 3 consecutive days. After an interval of several weeks they were reinoculated intravenously at weekly intervals with horse serum in amounts varying from 0.5 to 6 cc., or with egg-white in amounts varying from 0.4 to 0.8 cc. Lot C, consisting of 9 rabbits, was injected intravenously at weekly intervals with horse serum, egg-white, beef serum, sheep serum, dog serum, cat serum, human serum, pig serum, edestin, and casein. The edestin and casein were made up in 2 per cent solution in sodium carbonate. The proteins were given in doses of 1 cc. per kilo of body weight, the actual dose varying from 1 to 2.7 cc. It was so arranged that the total amount of serum, etc., which each rabbit in this group received was as great or greater than that given to the animals in Group B.

Finally, in Lot D, a series of 17 rabbits was given each a single dose of horse serum or egg-white, either intravenously or intraperitoneally, and killed at intervals varying from 3 to 29 weeks. 5 of these received 2 cc. of egg-white intraperitoneally. 4 received 2 cc. of horse serum intravenously, and 1 rabbit 1 cc. of horse serum intravenously; 2 rabbits received 2 cc. intraperitoneally, and 1 rabbit received 1 cc. intraperitoneally; 1 received 3 cc. and 1 received 5 cc. of sheep serum intravenously; 1 rabbit received 6 cc. of dog serum intravenously and 1 received 6 of cat serum intravenously.

In order to eliminate any possible effect which fresh serum might have, the sera for all these experiments were heated for one-half hour at 56° C.

Table I gives the results of these experiments.

TABLE I.

Animals and tissues examined.	Lot A. Normal control rabbits. Uninoculated.	Lot B. Rabbits sensitized to egg-white or horse serum and after 3 weeks reinjected with egg-white or horse serum.	Lot C. Rabbits receiving repeated inoculations of different proteins (horse serum, egg-white, beef, sheep, dog, cat, human, and pig serum, edestin, and casein).	Lot D. Rabbits receiving a single dose of foreign protein.
No. of rabbits	11	9	9	17
No. of intoxicating doses	0	2–10	1–10	1
Involvement of myocardium	0	9	3	7
Involvement of liver	0	9	1	4
Involvement of kidneys	1	9	3	12
Total No. of animals	11	9	9	17
Total No. positive results	1	9	3	12

The uninoculated control rabbits (Lot A) all showed normal organs except one. In this one rabbit a few areas of round cell infiltration were seen in the pelvis of the kidneys.

Of the eleven sensitized rabbits in Lot B, two died before intox-

icating doses of protein were administered. They showed normal organs. The nine sensitized rabbits receiving subsequent injections of serum all showed extensive and advanced changes in the heart muscle, liver, and kidneys.

In Lot C, only one-third of the animals developed changes in one or the other of these organs, and these were much less extensive than the lesions appearing in Lot B.

In Lot D, where the animals received but a single inoculation of egg-white or horse serum, the results corresponded quite well to those observed in Lot C.

It is, therefore, evident that though previous sensitization increases the incidence and severity of the changes, it is not necessary for their development.

During further experiments it was noted that when alterations occurred in the internal organs after a single inoculation they did not make their appearance for several days after inoculation. Experiments were, therefore, designed to determine more accurately the time of appearance of these lesions and to determine why this should be so long delayed. If the lesions were dependent upon the introduction of a toxic substance, one would expect, especially when the toxin was given in large amounts, that it would act much more promptly.

It is well known that the skin of an animal sensitized to a foreign protein such as horse serum will react specifically to the intracutaneous injection of that protein. In rabbits this specific sensitiveness of the skin usually develops within two to twenty days after the primary inoculation of horse serum, and, as Knox, Moss, and Brown[10] have shown, manifests itself after the intracutaneous injection of minute quantities of serum by the appearance in twelve to thirty-six hours of a large area of reddening, swelling, and edema, which persists at the site of inoculation for twelve to twenty-four hours. According to the view which generally prevails, the reaction is associated with the union of the antigen (horse serum) with antibodies formed against this specific protein.

This method was, therefore, utilized to determine whether or

[10] Knox, J. H. M., Moss, W. L., and Brown, G. L., *Jour. Exper. Med.*, 1910, xii, 562.

not the lesions in the internal organs following a single inoculation of horse serum were associated with the appearance of skin sensitiveness and, therefore, probably with the development of antibodies towards horse serum in the body of the animal.

It was at first hoped that repeated intracutaneous injections of horse serum in amounts varying from 0.02 to 0.1 cc. might be made, in order that the exact time at which sensitization developed could be fixed, but in normal rabbits used as controls the repeated intracutaneous injections, at four to six day intervals, of horse serum even in such small amounts led eventually to a positive reaction, which was interpreted as a true Arthus phenomenon. In such animals, however, general sensitiveness did not develop, since the injection of large quantities of horse serum intravenously in animals giving a positive skin test did not produce symptoms of anaphylaxis. In view of this fact it was possible to use the intracutaneous test only once.

For the final experiment 15 white rabbits were injected intravenously with from 5.5 to 9.0 cc. of horse serum in divided doses during 3 consecutive days.

TABLE II.

Association between the Development of Lesions in the Heart, Liver, and Kidneys and the Appearance of Skin Sensitiveness in 15 Rabbits Receiving Single Injections of Horse Serum.

Rabbit No.	Amount of serum.	Lived after injection.	Skin reaction.	Heart.	Liver.	Kidney.
	cc.	*days*				
15	5.5	7	Not made	0	0	0
9	8.5	8	" "	0	0	0
17	5.5	8	+	0	0	0
10	8.5	10	+	0	+	++
7	8.5	12	−	0	0	0
8	8.5	12	+	0	0	+
11	5.5	13	Not made	0	0	0
5	8.5	14	−	0	0	0
6	8.5	14	+	0	+?	0
13	5.5	17	+	0	0	+
16	5.5	21	−	0	0	0
18	5.5	22	− 20 hrs.	+	+	++
12	5.5	27	++	++	+	0
1	8.0	30	++	+	+	++
2	9.0	30	+	+	++	+
19 (control)	0			0	0	0
20 "	0			0	0	0
4 "	"		+ pure Arthus	0	0	0

At varying intervals after the first dose an intracutaneous injection of from 0.02 to 0.05 cc. of horse serum was made on one side of the shaved abdomen, while an equal amount of sheep or beef serum was injected in the skin of the opposite side as a control. The animals, except in one instance, were observed for from 36 to 48 hours and after the result of the skin test had been noted, killed, carefully autopsied, and their organs examined histologically.

The result of this experiment is given in Table II.

It will be seen that from the seventh to the twenty-first day after inoculation there were occasionally observed scattered and slight changes in the liver or kidney. When such alterations were found during this period they always occurred in rabbits that gave a positive skin test. All animals that gave a positive skin test, however, did not show changes in the internal organs.

After the twenty-second day the incidence and extent of the changes in the internal organs increased greatly and the skin reaction, except in one animal, was very intense. Uninoculated controls and one animal subjected to intracutaneous inoculation alone showed no changes in the internal organs.

Table II shows very well that the lesions in the internal organs appear either at the time the skin sensitiveness has made its appearance or directly afterwards, and therefore, after the animal has, hypothetically, produced antibodies to the foreign protein. One may liken the condition to serum disease in man during which time profound and very obvious disturbances may take place. In the rabbit the signs and symptoms so characteristic of the disease in man are lacking, though in one or two of the rabbits in this series moderate subcutaneous edema and generalized enlargement of the lymph nodes was observed at autopsy. Von Pirquet and Schick[11] have noted under the same conditions in rabbits alterations in the total and differential leucocyte counts that are analogous to those that are observed in children during serum sickness. The effect of anaphylactic shock is, however, so unlike in different species of animals that it is not permissible to draw general conclusions from the results obtained in any one species.

[11] von Pirquet, C. F., and Schick, B., Die Serumkrankheit, Leipzig and Vienna, 1905.

CONCLUSIONS.

Such foreign proteins as horse serum and egg-white in the amounts employed in these experiments do not produce evidences of intoxication immediately after injection into rabbits. Single large injections do, however, produce changes in the parenchymatous organs after a period of ten to twenty-one days. These develop at the time or immediately after the animal has formed antibodies for the foreign proteins.

The mechanism of the development of the lesions in the myocardium, liver, and kidneys of rabbits is thus the same, whether a single inoculation is given or whether repeated inoculations are made in sensitized animals. By the latter method, however, much more marked and extensive changes may be produced.

THE EFFECT OF ROENTGEN RAYS ON THE RATE OF GROWTH OF SPONTANEOUS TUMORS IN MICE.

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In a previous communication we reported the existence of a relationship between the resistant state to transplantable tumors in mice and a lymphocytic crisis in the circulating blood. We further demonstrated that by a previous destruction of the lymphoid tissue of these animals with x-ray a potentially resistant animal was rendered susceptible to cancer inoculation.[1]

In the course of some experiments on x-ray we have noted that the lymphoid elements, after extensive depletion by x-ray,[2] will soon start to regenerate actively. This process will continue, as has been noted before, to a period of overproduction of the lymphoid elements. The rapidity with which this occurs depends somewhat on the amount of original destruction and somewhat on the general condition of the animal. We have further noted that by one small dose of x-ray we could obtain in a certain proportion of animals a stimulation of the lymphoid elements, preceded by a comparatively short period in which the lymphocytes were below normal. This suggested an explanation of certain therapeutic effects of x-ray.

Our first problem was to determine whether or not x-rays in a small dose administered to an animal as a whole would produce an effect on the subsequent growth of a cancer, different from that produced by a similar dose applied directly to the cancer outside the body. For this purpose it was necessary in one set of animals to confine the x-ray effect to the animal alone, ruling out any possible action on the cancer, and, in a second set to confine the x-ray effect to the cancer, preventing an indirect effect on the animal. Spontaneous tumors of the mouse were selected for this work as a more

[1] Murphy, Jas. B., and Morton, J. J., *Jour. Exper. Med.*, 1915, xxii, 204.
[2] Heineke, H., *Mitt. a. d. Grenzgeb. d. Med. u. Chir.*, 1905, xiv, 21.

suitable material than the transplanted tumors, for reasons that will be explained later. The results are given here in a brief summary. A complete analysis of the size and characters of the cancers with autopsy findings will be given in a subsequent report.

Series I.—There were 52 mice with various stages and types of spontaneous cancers in this group. The tumors were removed as completely as possible by operation, and, with the cancer out, the whole animal was exposed to a stimulating dose of x-ray (Coolidge tube). Immediately afterwards a graft of the original cancer was replaced in the groin of the animal. In 26 of the 52 animals treated in this fashion, there resulted a complete immunity to the recurrence of the disease. Only those animals were included in this number that lived and remained in good physical condition for at least five weeks after the treatment. The majority lived from two to four months, some to eight months, and some are still living. There has been no evidence of a local recurrence at the site of operation, nor where the graft was implanted, or of metastasis in those that have died. Among the remaining 26 animals of the series the average time for the appearance of the graft was five weeks and four days, a figure which contrasts strongly with the figure for the control animals. The number of recurrences at the original location of the tumor was 11 among the 52 animals, all occurring in the latter 26.

Series II.—For a control series we had 29 mice with spontaneous tumors of various sorts. These were operated on in the same manner as the animals in the first series, but a graft of the cancer was returned without treatment to either the animal or the cancer. The tumors were kept outside the body for the same length of time as in the first series. In 28 of the 29 the grafts grew progressively. In one the graft grew for a period and then retrogressed to complete absorption. The average time for the grafts to become palpable was one week and five days. This is about the same figure obtained by Rous.[3] Local recurrences of the cancer occurred in 14 of the 29 animals.

Series III.—The cancers in these animals were removed in the same manner as in the first two series, but in this group the cancers were subjected to the same amount of treatment that the animals had

[3] Rous, P., *Jour. Exper. Med.*, 1914, xx, 433.

received in the first series. A graft from the cancer, after this treatment outside of the body, was returned to the groin of the original host, as in the other experiments. 10 mice with spontaneous tumors were used for this series, and in all 10 the returned grafts grew. The average time for these to become palpable was one week and three days. There was a local recurrence of the tumor in 4 of the 10 animals.

A tabulation of the figures for the three experiments is given for comparison in Table I.

TABLE I.

	Immune.	Susceptible.	Local recurrence of tumor.	Average time for appearance of graft.
	per cent	*per cent*	*per cent*	
Series I*.....	50.0	50.0	21.2	5 wks., 4 days.
" II.....	3.4	96.6	48.3	1 wk., 5 "
" III.....	0.0	100	40.0	1 " 3 "

* Series I, animals treated by x-ray, while cancer was out. Later a graft of the tumor was returned. Series II, control animals in which cancer was removed and a graft returned without treatment to either animal or tumor. Series III, cancers removed and subjected direct to x-ray treatment and a graft returned to the original host.

It will be seen from the figures in Table I that x-ray administered directly to the cancer outside of the body is insufficient to prevent the growth of a graft returned to the original host. On the other hand, the same small dose of x-ray given to the animal with the cancer removed was sufficient to render 50 per cent of the mice so treated immune to returned untreated grafts of their own tumors and greatly retard the growth in the other 50 per cent.

The contrast between the control animals with 1 immune out of 29, and the x-rayed series with 26 immune out of 52 is striking, as is also the comparison of local recurrence in the two series. The delayed appearance of the graft in the treated series is important and this period could perhaps have been prolonged or a recurrence prevented altogether by a second exposure to x-ray after a suitable interval.

Total white counts and differentials were done on all of these mice

before operation[4] or treatment and on part of them at intervals after. So far in the limited number counted systematically afterward, our treatment has given in the successful cases a definite increase in the lymphocytes. Whether or not this increase is vitally concerned in the immunity process to spontaneous tumors is a point to which at present we are unwilling to commit ourselves. The results in the light of our previous experiments are strongly suggestive of this, however.

We have demonstrated a direct effect of x-rays on the animal, which renders it more highly resistant to replants of its own cancer than would normally be the case. There is also an absence of any demonstrable effect of this small dose of x-ray when administered to the cancer direct. Grafts of such tumors when returned to their original host grow as well as do the controls.[5]

[4] Counts made on over 100 untreated mice with spontaneous tumors have failed to show an abnormally low lymphocytic content in the circulating blood. This result is contrary to that obtained by Baeslack (Baeslack, F. W., *Ztschr. f. Immunitätsforsch., Orig.*, 1913–14, xx, 421) in counts on two mice with spontaneous tumors. We were also unable to confirm his reported decline in the numbers of lymphocytes in animals with growing transplantable cancers (Murphy and Morton, *loc. cit.*). He also gives differential counts on four mice with natural immunity to transplanted tumors, but as no total white cell counts are given it is impossible to tell whether his percentage variations are due to fluctuations in the polymorphonuclear cell or in the lymphocyte.

[5] We have avoided in this communication any discussion of the massive and contradictory literature on direct x-ray effects. We are unaware of any experiments that bring out the above points.

A FURTHER STUDY ON THE BIOLOGIC CLASSIFICATION OF PNEUMOCOCCI.

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(Received for publication, September 17, 1915.)

The biologic classification of pneumococci, according to Dochez and Gillespie,[1] divides these organisms into four main groups. These groups represent well defined types which are distinct and readily differentiated by immunological reactions. In previous communications[2] the varieties of pneumococci, their relation to disease, and their significance in problems of specific therapy have been discussed in detail. The present work is confined to a study of a limited number of strains of pneumococci which, because of certain serological reactions, are closely allied to the second group, and appear to represent distinct subgroups of Pneumococcus Type II.

In classifying pneumococci by serological methods, agglutination and protection experiments have been employed. Agglutination of typical pneumococci of the second group in an immune serum of the homologous type is prompt and characteristic. There is occasionally encountered, however, an organism with which the agglutination reaction in Antipneumococcus Serum II is incomplete and less prompt, often being delayed several hours. The occurrence of strains showing these peculiarities has been frequent enough to direct attention to them, since in the presence of a positive, but atypical agglutination, some confusion may arise in diagnosis. Theoretically, too, it is of interest to determine, if possible, whether or not these variations in agglutinability indicate essential biologic differences.

The facts presented are a result of a study of ten strains of pneumococci, all of which agglutinated in Antipneumococcus Serum II, atypically as described above. Cross-immunity reactions and

[1] Dochez, A. R., and Gillespie, L. J., *Jour. Am. Med. Assn.*, 1913, lxi, 727.

[2] Cole, Rufus, *Arch. Int. Med.*, 1914, xiv, 56; *New York Med. Jour.*, 1915, ci, 1, 59. Dochez, A. R., and Avery, O. T., *Jour. Exper. Med.*, 1915, xxi, 114.

absorption tests indicate that these organisms possess characteristic group relationships among themselves and a common antigenic relation to the original and typical Type II pneumococcus.

Description of Cultures.—Ten strains of pneumococci were chosen, five of which were isolated from disease and five from the sputum of normal individuals. Table I gives the source and culture designation of the strains studied.

TABLE I.

Pneumococcus.	Source.	Obtained from	Remarks.
J	Acute maxillary antrum	Pus	Recovery.
L	" lobar pneumonia	Lung puncture	"
M	" " "	Blood	Septicemia, fatal.
Jn	" " "	Sputum	Recovery.
W	Primary pneumonia (child)	Lung puncture	Fatal.
As	Normal individual	Sputum	No infection.
Ar	" "	"	" "
F C B	" "	"	" "
S 13	Diabetic patient	"	" "
H	" "	"	" "

These ten strains possess the common characteristic of partial agglutination in antipneumococcus serum of Type II. Culturally and biologically they present all the usual characters of typical pneumococci: such as inulin fermentation, bile solubility, and more or less distinct capsule formation. Their virulence on isolation was distinctly lower than that of the typical Type II organism.

Agglutination as illustrated in Table II establishes a definite relationship between the strains studied and the type organism of Pneumococcus Group II. The reaction of all ten strains in Serum II is distinct, but never as prompt or complete as in the case of the typical Group II organism. With all typical Type II pneumococci the agglutination begins almost immediately and is complete in half an hour, while the reaction of these ten strains is always delayed and often incomplete at the end of the period of observation. It is further evident that these minor agglutinins tend to disappear in the higher dilution and are completely absent in dilution of 1 to 80, at which titer the reaction of the type organism still persists.

This variation in agglutination was at first attributed to the possibility that there might exist, among pneumococci of Group II,

EXPERIMENTAL.

Agglutination Experiments.

TABLE II.

Determination of the Titer of Agglutination of Strains in Antipneumococcus Serum II, Using Antipneumococcus Serum I and Normal Horse Serum (N) as Controls.

Dilution.	1:1			1:10			1:20			1:40			1:80		
Sera.	N	I	II	N	I	II	N	I	II	N	I	II	N	I	II
Culture															
As 11^{5}	−	−	++	−	−	++	−	−	++	−	−	−	−	−	−
L 6^{8}	−	−	++	−	−	++	−	−	++	−	−	+	−	−	−
Jn 8^{9}	−	±	++	−	−	++	−	−	++	−	−	++	−	−	−
M 5^{10}	−	−	++	−	−	++	−	−	++	−	−	+	−	−	−
Ar 9^{5}	−	±	++	−	−	++	−	−	++	−	−	+	−	−	−
J 7^{8}	−	−	++	−	−	++	−	−	++	−	−	+	−	−	−
W 5^{9}	−	−	++	−	−	++	−	−	++	−	−	±	−	−	−
F C B 9^{5}	−	+	++	−	±	+	−	−	−	−	−	−	−	−	−
S 13 12^{3}	−	+±	++	−	−	++	−	−	+	−	−	−	−	−	−
H 7^{3}	−	+	++	±	−	+	−	−	+	−	−	±	−	−	−
II 46^{11}	−	−	++	−	−	++	−	−	++	−	−	++	−	−	++
I 115^{3}	−	+	−	−	+	−	−	+	−	−	+	−	−	±	−

The reactions were read after 2 hrs. at 37° C. and over night on ice.

The numerals following the culture indicate the animal passage; the exponent indicates the number of generations removed from the last passage.

TABLE III.

The Effect of Increased Virulence upon the Agglutinability of the Atypical Group II Pneumococcus in Antipneumococcus Serum II.

Antipneumococcus Serum II.	Dilution.			
	1:10	1:20	1:40	1:80
Pneumococcus				
M 0^{9}	++	++	±	−
M 5^{11}	++	++	±	−
L 0^{9}	++	++	−	−
L 6^{11}	++	++	±	−
Jn 0^{9}	+	±	−	−
Jn 10^{3}	++	+	−	−
F C B 1^{16}	++	−	−	−
F C B 11^{3}	++	−	−	−
II 46^{11}	++	++	++	++

The numerals after the culture indicate number of mouse passages.

The exponent indicates the number of generations removed from the last passage.

strains of poor agglutinability, analogous to similar conditions among other bacterial groups. It was also thought that, since these organisms were of lower virulence than the type organism, this fact might bear some relation to their agglutinability. Agglutination tests were carried out with certain atypical strains, the virulence of which had been enhanced by repeated animal passage. The effect of the animal passage on the agglutination titer was then determined. Four strains were chosen, the virulence of which was raised by mouse passage until it had attained a point comparable to that of the typical Type II pneumococcus, with a minimum lethal dose of 0.000001 cc. of broth culture.

It is evident from Table III that enhancing the virulence of these strains did not affect their agglutinability. The variations in agglutinations between these strains and the type pneumococcus appear to be not merely differences in agglutinability, but suggest rather the possibility that actual differences in agglutinogenic properties may characterize these organisms. To determine this a univalent immune serum was prepared by immunization of rabbits to each of the ten strains.

Agglutination by Immune Rabbit Sera of the Ten Strains of Atypical Group II Pneumococci and the Results Obtained by Cross-Agglutination.

TABLE IV.

Agglutination of the Ten Strains by Homologous Sera and the Effect of Such Sera on Stock Cultures of Pneumococcus of Type II.

Immune rabbit sera.

Sera.	As		Ar		S 13		F C B		H	
Culture.	As	II	Ar	II	S 13	II	F C B	II	H	II
1 : 1	++	−	++	−	++	−	++	−	++	−
1 : 10	++	−	++	−	++	−	++	−	++	−
1 : 20	++	−	+	−	++	−	++	−	++	−
1 : 40	++	−	±	−	++	−	±	−	++	−

Sera.	L		J		Jn		W		M	
Culture.	L	II	J	II	Jn	II	W	II	M	II
1 : 1	++	−	++	−	++	−	++	−	++	−
1 : 10	++	−	++	−	++	−	++	−	+±	−
1 : 20	++	−	++	−	++	−	++	−	+±	−
1 : 40	++	−	++	−	++	−	++	−	+	−

Table IV shows that the rabbit immune atypical sera each agglutinated strongly the homologous strain of pneumococcus, but that none of these sera had any effect on the type culture of Group II. This failure of the antisera of the ten strains to agglutinate the Type II pneumococcus shows differences in the antigenic properties of these organisms and is in striking contrast to the positive reactions of agglutination with these same strains by Antipneumococcus Serum II (Table II). This failure of reversibility of the agglutination reaction would seem, therefore, to be due to actual differences in the agglutinogenic groups of these various organisms.

TABLE V.

Cross-Agglutination.

Immune sera.	Pneumococcus cultures.										
	As	S 13	F C B	Ar	L	J	Jn	W	M	H	II
As	++	−	−	−	++	−	++	−	++	−	−
S 13	−	++	−	−	−	−	−	−	−	−	−
F C B	−	−	++	−	−	−	−	−	−	−	−
Ar	−	−	−	++	−	+	−	+	−	−	−
L	++	−	−	−	++	−	++	−	++	−	−
J	−	−	−	++	−	++	−	++	−	−	−
Jn	++	−	−	−	++	−	++	−	++	−	−
W	−	−	−	++	−	+	−	++	−	−	−
M	+	−	−	−	+	−	+	−	+	−	−
H	−	−	−	−	−	−	−	−	−	++	−
Normal rabbit	−	−	−	−	−	−	−	−	−	−	−
II	+	++	+	+	++	+	++	++	++	++	++

Table V reveals the striking fact that in a series of organisms wholly contained within Group II certain separate relationships are demonstrable. An analysis of this table indicates clearly the existence of three distinct groups which appear to represent subdivisions of the main Group II, in the antiserum of which all members of each of these subdivisions are agglutinated. For purposes of convenience these subdivisions will be referred to as Subgroups II A, II B, and II X, and are shown more clearly in Table VI.

Subgroup II A consists of four strains, the immune reactions of which are specific within the group, being identical with those of all other strains of the group, but bearing no relation to those of Subgroups II B, or II X. Similarly, Subgroup II B consists of three other strains characterized by the possession of specific immunity

TABLE VI.

Subgroup II A.

Immune sera.	Pneumococcus cultures.				
	As	L	Jn	M	II
As	++	++	++	++	−
L	++	++	++	++	−
Jn	++	++	++	++	−
M	++	+	+	+	−
II	+	++	++	++	++

Subgroup II B.

Immune sera.	Pneumococcus cultures.			
	Ar	J	W	II
Ar	++	+	+	−
J	++	++	++	−
W	++	+	++	−
II	+	+	++	++

Subgroup II X.

Immune sera.	Pneumococcus cultures.			
	F C B	S 13	H	II
F C B	++	−	−	−
S 13	−	++	−	−
H	−	−	++	−
II	+	++	++	++

reactions, identical for members of this subgroup alone. The remaining three strains have been placed in Subgroup II X which, like the larger Group IV of the original biologic classification, is peculiar in that it seems to consist of a heterogeneous series of independent strains which do not cross in their immunity reactions with members of the other two subgroups or with each other. All, however, possess the common character of atypical agglutination in Antipneumococcus Serum II. This subgroup, like its prototype Group IV, seems to be infinitely variable, and to be characterized by the absence of cross-immunity reactions and by lower virulence.

Protection Experiments.—The protection of animals against infection is generally conceded to be one of the most specific of immunological reactions and hence one of the most satisfactory methods of classification. White mice were given intraperitoneally graduated doses of pneumococci and at the same time a fixed quantity of immune serum. All animals except the virulence controls received 0.2

cc. of immune serum intraperitoneally. This quantity of Immune Serum II as a rule protects mice against 0.01 cc. of broth culture of the homologous organism which, given alone, kills mice regularly in doses of 0.000001 cc. All animals surviving for five days were considered effectively protected.

In experimental pneumococcal infection the specificity of the protective power of an immune serum is evident only when the culture employed is fully virulent. Of the ten strains of pneumococci it was found possible to raise the virulence of seven by animal passage. The virulence of the other three strains could not be increased sufficiently for use in protection experiments, although they were passed successively through 11, 14, and 18 animals, respectively. They were even then of such low virulence that it was impossible to kill mice with the moderate doses necessary for the successful carrying out of the test. Of these three strains, two belonged to Subgroup II X and one to Subgroup II A.

Further Evidence of the Specificity of Group Relationships by Protection Experiments.

TABLE VII.

Protective Action of Antipneumococcus Serum II.
The Relation between the Specificity of the Protective Action of Immune Serum and the Virulence of the Infecting Organism.
Pneumococcus L (Subgroup II A) after One Animal Passage.

Pneumococcus L1[1].	Virulence controls.	Serum II.	Serum I.	Normal serum.
cc.				
0.01		S.*	D. 96	S.
0.001	D. 72	"	S.	"
0.0001	S.	"	"	D. 96
0.00001	"	"	"	S.
0.000001	D. 72	"	D. 96	D. 96

Pneumococcus L (Subgroup II A) after Seven Animal Passages.

Pneumococcus L 7[1].	Virulence controls.	Serum II.	Serum I.
cc.			
0.01		S.	D. 18
0.001		"	" "
0.0001	D. 36	"	" 24
0.00001	" "	"	" 36
0.000001	" "	"	" "

* In the tables D. stands for died; S. for survived. The figures represent the number of hours before the death of the animal.

Table VII shows that after increasing the virulence of pneumococcus L (Subgroup II A) to a degree sufficient to apply the test, definite protection was afforded by stock Antipneumococcus Serum II against 10,000 times the minimal lethal dose of culture. The odd survivals and non-specific reactions with the same strain before its virulence was raised by animal passage is evidence of the futility of attempting specific reactions of a protective nature with avirulent organisms. That this phenomenon does not represent a reversion to type brought about by animal passage is evidenced by the fact that the antigenic properties of these organisms remained unaffected by such treatment. Of the seven strains, the virulence of which was increased by mouse passage, Antipneumococcus Serum Type II protected against six, three of which belonged to Subgroup II A and three to Subgroup II B. The seventh strain, an organism of Subgroup II X, although made equally virulent by nine mouse passages, was not protected against by immune serum of Type II.

Cross-Protection Tests with Homologous and Heterologous Sera of the Three Subgroups of Pneumococcus II.

TABLE VIII.

Protective Action of Sera of Subgroup II A against a Pneumococcus of the Same Group, and Failure of Sera of Subgroups II B and II X to Protect against the Same Organism.

Pneumococcus Subgroup II A.	Immune sera.					
	Subgroup II A.		Subgroup II B.	Subgroup II X.	Antipneumococcus Type II.	Virulence controls.
Jn 91	Jn	L	W	F C B		
cc.						
0.01	D. 72	S.	D. 18	D. 18	D. 96	
0.001	S.	"	" "	" "	S.	
0.0001	"	"	" "	" "	"	D. 18
0.00001	"	"	" "	" 24	"	" "
0.000001	"	"	" "	" "	"	" 24

Tables VIII and IX demonstrate that an immune serum of Subgroups II A and II B protects against any organism of the homologous subgroup, but fails to protect against any strain of the other two subgroups. Table X emphasizes the individual character of organ-

TABLE IX.

Protective Action of Sera of Subgroup II B against a Pneumococcus of the Same Subgroup and Failure of Sera of Subgroups II A and II X to Protect against the Same Organism.

Pneumococcus Subgroup II B.	Immune sera.					
	Subgroup II B.		Subgroup II A.	Subgroup II X.	Antipneumococcus Type II.	Virulence controls.
W 61	W	J	Jn	F C B		
cc.						
0.01	D. 26	S.	D. 18	D. 18	D. 96	
0.001	S.	"	" "	" "	S.	
0.0001	"	"	" "	" "	"	D. 18
0.00001	"	"	" "	" "	"	" "
0.000001	"	"	" "	" "	"	" "

TABLE X.

Protective Action of Serum of Subgroup II X against the Homologous Organisms Only and Failure of Sera of Subgroups II A and II B and Antipneumococcus Serum II To Protect against the Same.

Pneumococcus Subgroup II X.	Immune sera.					
	Subgroup II X.		Subgroup II A.	Subgroup II B.	Antipneumococcus Type II.	Virulence controls.
F C B II1	F C B	S 13	Jn	W		
cc.						
0.01	D. 18	D. 18	D. 18	D. 18	D. 18	
0.001	S.	" "	" "	" "	" "	
0.0001	"	" "	" "	" "	" "	D. 18
0.00001	"	" "	" 22	" "	" "	" "
0.000001	"	" "	" "	" 36	" "	" "

TABLE XI.

Lack of Protective Power of Immune Sera of Subgroups II A, II B, and II X against Typical Pneumococcus II.

Pneumococcus Type II.	Immune sera.			
	Subgroup II A.	Subgroup II B.	Subgroup II X.	Virulence controls.
II 461	Jn	W	F C B	
cc.				
0.01	D. 18	D. 18	D. 18	
0.001	" "	" "	" "	
0.0001	" "	" "	" "	D. 18
0.00001	" "	" "	" "	" "
0.000001	" "	" "	" 26	" "

isms of Subgroup II X. A serum produced by immunization with any given strain of this type protects against that particular organism and against no other. As previously noted, there is a complete lack of crossing in the immunity reactions of the individual members of Subgroup II X. Table XI shows that in protective action, as in agglutination (Table IV), the immune sera of Subgroups II A, II B, and II X have no effect on the typical pneumococcus of Type II. That these immunologic reactions between the original Type II pneumococcus and organisms of the subgroups are not reversible seems to indicate degrees of difference in antigenic characters. While the serological specificity of the subgroups definitely separates one from the other, nevertheless their immune reactions with the antisera of the typical Type II organism indicate that they are all biologically related. The correlation of these subgroups is further proven by absorption tests.

Absorption Experiments.—The phenomenon of specific absorption of agglutinins from an immune serum by the homologous organism was first described by Castellani.[3] This investigator found that from a polyvalent serum produced by immunization with two microorganisms of different species, the agglutinins for either one could be removed by fractional absorption with the homologous strain, while in a serum thus exhausted, the antibodies for the second organism remained intact. It has been shown also that in a univalent serum against *Bacillus typhosus* not only are agglutinins present for that organism alone, but that in the same serum there also exist partial or minor agglutinins for bacilli which are biologically similar, and which fall within the same general group. Absorption of a typhoid immune serum by *Bacillus typhosus* removes not only the agglutinins for that organism, but completely exhausts the serum of its minor antibodies for the closely allied organisms. This reaction, however, is not reversible, for removal of the partial agglutinins by absorption with a member of the intermediary species leaves the antibodies for *Bacillus typhosus* practically undiminished. The significance of this phenomenon in bacterial classification is obvious, and its applicability to the present study is evident in the following protocols.

[3] Castellani, A., *Ztschr. f. Hyg. u. Infectionskrankh.*, 1902, xl, 1.

Specificity of Absorption Reaction. Absorption of Antipneumococcus Serum I with Pneumococci of Groups I and II.

Technique.—Specific absorption. Antipneumococcus Serum I. 2 cc. of serum were diluted with 3 cc. of salt solution. To the 5 cc. of diluted serum the live, washed bacterial residue of 150 cc. of a twenty-four hour broth culture of Pneumococcus I was added, allowed to stand in contact over night in the ice box, then centrifuged, the clear supernatant serum pipetted off, and passed through a Berkefeld filter.

Non-specific absorption. Antipneumococcus Serum I was absorbed with Pneumococcus II. The technique was the same as above.

Control. Antipneumococcus Serum I diluted, and, without the addition of any bacteria, filtered by the same technique.

TABLE XII.

Specific and Non-Specific Absorption of Agglutinins from Antipneumococcus Serum I by Pneumococci of Groups I and II.
Agglutination.

Culture pneumococcus, Group I.	Antipneumococcus Serum I.		
	Specific absorption with Pneumococcus I.	Non-specific absorption with Pneumococcus II.	Control. Serum unabsorbed.
	−	++	++

TABLE XIII.

Protective Power of Antipneumococcus Serum I after Absorption with Pneumococci of Groups I and II.
Protection.

Culture pneumococcus, Group I.	Virulence controls.	Antipneumococcus Serum I.		
		Specific absorption with Pneumococcus I.	Non-specific absorption with Pneumococcus II.	Control. Serum unabsorbed.
cc.				
0.01		D. 18	S.	S.
0.001		" 20	"	"
0.0001	D. 18	" 24	"	"
0.00001	" "	" "	"	"
0.000001	" 20	" 48	"	"

Tables XII and XIII demonstrate the specificity of the absorption reaction with pneumococci of the fixed Types I and II. Saturating an immune serum of Group I with pneumococcus of the same type completely exhausts that serum of all its agglutinins and protective antibodies, while absorption of the same serum with organisms of Group II does not appreciably diminish these immune substances for pneumococci of Group I.

Absorption of Antipneumococcus Serum II with Pneumococcus II and Organisms of Its Subgroups II A, II B, and II X.

Technique.—To 10 cc. of undiluted Antipneumococcus Serum II was added the washed bacterial residue from 150 cc. of an eighteen hour broth culture of the given strain of pneumococcus. Before being added to the serum the bacteria were killed by heating at 56° C. for forty-five minutes. The serum mixtures were incubated in the water bath for two hours at 37° C. and allowed to remain in contact over night in the ice box. The clumps of agglutinated bacteria were whirled out by centrifugation, and the clear supernatant serum was pipetted off. This serum was absorbed a second time by the same technique, tested for the absence of agglutinins, and called exhausted serum.

TABLE XIV.

Cross-Agglutination Reactions with Antipneumococcus Serum II Exhausted by Absorption with Type Strains of Pneumococcus II and Its Subgroups II A, II B, and II X.

Antipneumococcus Serum II. Absorbed by	Pneumococcus.										
	Subgroup II A.				Subgroup II B.			Subgroup II X.			Group II.
	Jn	As	L	M	W	Ar	J	S 13	F C B	H	Type II.
Subgroup A, Jn	−	−	−	−	++	+±	++	++	+	++	++
" B, W	++	++	++	+±	−	−	−	++	+	++	++
" X, S 13	++	++	++	+±	++	++	++	−	+	++	++
Group II	−	−	−	−	−	−	−	−	−	−	−

Table XIV shows that specific absorption of Antipneumococcus Serum II with the typical Type II pneumococcus removes all the agglutinins, not only for the homologous organism, but also all the partial agglutinins for its subgroups, II A, II B, and II X. In other words, specific absorption of Antipneumococcus Serum II completely exhausts it of both major and minor agglutinins. Conversely, however, absorption of the same immune serum with a representative strain of Subgroup II A removes the minor agglutinins for members of that subgroup only, leaving intact the antibodies for the Type II pneumococcus and its other subgroups, II B and II X. Similarly, absorption of Antipneumococcus Serum II with any member of Subgroup II B takes out the agglutinins for all the Subgroup II B organisms, but leaves unaffected the antibodies for the Type II pneumococcus and its subgroups, II A and II X. The lack of cross-immunological reactions among the heterogeneous organisms within

Subgroup II X already noted in the previous agglutination and protective experiments (Tables VI and X) is again evident in the absorption tests. Saturation of Immune Serum II with a pneumococcus of Subgroup II X robs the serum of its agglutinins for that individual strain only, and for no other.

The results obtained by absorption experiments with Antipneumococcus Serum II, a serum produced by intensive immunization of the horse with a single strain of the typical Type II pneumococcus, corroborate the same specific groupings obtained by the cross-immunological reactions of agglutination and protection with immune rabbit sera of the individual strains.

DISCUSSION.

The biologic classification of the pneumococcus distinguishes four distinct groups. These types are based upon well defined immunologic differences. The accuracy with which these groups may be differentiated and the constancy of their relative frequence in disease and health emphasize the importance of their recognition in clinical and epidemiological studies. The exactness with which the large number of strains studied have conformed to type indicates the extraordinary uniformity and comparative fixity of the specific groups. These distinctive differences in antigenic properties not only offer a reliable method for the more exact determination of the varieties of pneumococcus, but afford the only rational basis for the study of immunotherapy in pneumococcal infection.

The second group of pneumococci of the original classification consists of highly virulent organisms which are responsible for about one-third of all cases of lobar pneumonia of pneumococcus origin. Organisms of this group produce infections which are clinically severe, and the mortality of which is about 35 per cent. The serological reactions by means of which Group II was originally identified are sharply defined. So characteristic and prompt is the agglutination of any strain of this type in Antipneumococcus Serum II that any deviation from the normal reaction is quickly recognized. The isolation of an occasional strain of pneumococcus which agglutinates atypically in Antipneumococcus Serum II led to an attempt to determine the nature of these organisms and their relation to the

type pneumococcus of Group II. Of the ten strains studied five were isolated from disease and five from the sputum of normal individuals. These organisms exhibited the usual cultural and biochemical characteristics of pneumococcus; namely, inulin fermentation, bile solubility, and varying degrees of capsular development. The facts developed by this study indicate the existence of pneumococci which are biologically similar and closely allied to the typical organism of Group II. These organisms possess partial antigenic characters common to the Type II pneumococcus, but they vary from the typical representative of this group by a diversity of relationships among themselves, and by a lack of the reversibility of their immune reactions with the type organism. Because of these variations these organisms may be classified as subvarieties of Pneumococcus Group II. All strains of the three subgroups thus far recognized are agglutinated by Antipneumococcus Serum II, but the diminished intensity of the reaction serves to distinguish them from typical II pneumococci. The incomplete reaction of agglutination of these subvarieties in the immune serum of Type II is apparently similar to the diminished reactions which occur in many immune sera with other organisms closely allied to the type used in producing the serum. Such reactions occur only in the higher concentrations of immune sera and may be attributed to the so called minor agglutinins. In such cases, also, a non-reversibility of the immune reaction has been noted. For instance, a potent typhoid immune serum may agglutinate *Bacillus coli* in the higher concentrations, but an anticolon serum may not affect *Bacillus typhosus*. All strains of these subgroups of pneumococci are partially agglutinated in the higher concentrations of Antipneumococcus Serum II, while conversely the immune serum produced by any strain of these subgroups fails to react with the typical II pneumococcus.

In addition to the partial agglutination of these strains in Antipneumococcus Serum II and the absence of reverse immunity reactions, these subvarieties are further characterized by certain interrelationships of a definite antigenic nature, by virtue of which they may be classified into at least three subgroups, which have been called Subgroups II A, II B, and II X. It has been shown in the preceding protocols that a given member of either Subgroup II A or

II B is characterized by the possession of immunity reactions identical with those of all other strains of the homologous subgroup, and that these reactions are specific only within the group. Subgroup II X is peculiar in that it seems to consist of a heterogeneous series of independent strains which do not cross in their immunity reactions with members of the other two subgroups or with each other. This subgroup, like its prototype, Group IV, of the original biologic classification, is of lower virulence, infinitely variable in its composition, and lacking in cross-immunity reactions. As has been noted, serum Type II fails to protect against organisms of this subgroup, and inasmuch as specific protection is regarded as the ultimate criterion for classification, it is doubtful whether organisms of Subgroup II X are of sufficiently close relationship to be included within Group II.

The specificity of these subgroups, II A and II B, as tested by the immunity reactions of agglutination and protection is further confirmed by the phenomenon of absorption. Saturation of Antipneumococcus Serum II with a typical Group II pneumococcus removes all the agglutinins both for the type organism and its three subgroups. Absorption of the same serum, however, with a member of either Subgroup II A or II B removes only the partial agglutinins for the homologous subgroup, but leaves intact the antibodies for the typical II pneumococcus and the other subgroup. Absorption of Antipneumococcus Serum II, on the other hand, by any member of Subgroup II X takes out the antibodies for that particular strain only, and for no other.

In the present discussion no attempt is made to interpret the experimental data in terms of their phylogenetic significance. Whether the subvarieties of the second group of pneumococci represent strains which have acquired independently certain adaptive characters, or whether they are related to each other and to the fixed type by the lineage of common descent is interesting. However, the limited nature of the present study precludes the formulation of any hypothesis as to origin.

SUMMARY.

1. At least three subgroups of Pneumococcus Type II may be recognized by specific immune reactions. They have been called Subgroups II A, II B, and II X.

2. That the organisms of these three subgroups are biologically related to Pneumococcus Type II is shown by the following facts: (a) Agglutination with Antipneumococcus Serum II. (b) Protection with Antipneumococcus Serum II, except Subgroup II X. (c) Absorption of Antipneumococcus Serum II with typical Type II pneumococcus removes the antibodies for all subgroups. (d) Absorption of Antipneumococcus Serum II with a member of Subgroups II A or II B removes only the antibodies for the homologous subgroup. Absorption of Antipneumococcus Serum II with any given member of Subgroup II X removes the antibodies for that particular strain only.

3. That the three subgroups, although biologically related to Pneumococcus Type II, possess, nevertheless, specific differential characters which separate them one from another, is evidenced by the following facts: (a) The organisms of any subgroup are not agglutinated by the antisera of the other two subgroups. (b) They are not protected against by the sera of the other subgroups. (c) They do not absorb from Antipneumococcus Serum II the specific immune bodies of the other subgroups.

4. Subgroups II A and II B are characterized by immunity reactions identical within the respective group.

5. Subgroup II X consists of heterogeneous strains which do not cross in their immunity reaction with each other or with Subgroups II A or II B.

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SPONTANEOUS AND EXPERIMENTAL LEUKEMIA OF THE FOWL.

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During the past three years a transmissible leukemia of the fowl has come under observation, and, in the following paper, a summary of the literature, together with a brief presentation of the clinical and anatomical aspects of the disease will be given.[1]

HISTORICAL.

Moore (1) reported infectious leukemia in fowls, but this has since been regarded as not true leukemia. Butterfield (2) and Mohler[2] recognized the condition from the postmortem findings, and the first careful study, including the clinical course and anatomical changes, was made by Warthin (3) in 1907. The predominating cell in the blood and tissues was the large lymphocyte,[3] and the condition was diagnosed leukemic lymphocystostoma. Other cases described by both Butterfield and Warthin lacked the blood changes but showed the same tissue picture and were considered by these authors as examples of aleukemic leukemia.

Kon (4) and later Soshestrenski (5) each report a similar case of leukemia. They consider their case as true splenic leukemia.

Ellermann and Bang (6, 7), in 1908, were the first to transmit the disease successfully from a spontaneous case to other healthy fowls. They report two typical spontaneous cases in every respect similar to Warthin's first case. They transmitted the disease by inoculation of organic emulsion through three generations, producing blood picture and organic findings identical with the spontaneous cases. They also found by inoculation of organic extract from a pseudoleukemic fowl (used in the same sense as aleukemia of Warthin) that a picture of true leukemia resulted, and concluded that the two conditions are etiologically identical.

In several subsequent reports (8, 9) Ellermann and Bang call attention to the following points: (1) that in transmitted cases the disease may appear as

[1] A more detailed and fully illustrated study will appear in the *Rep. Johns Hopkins Hosp.*, 1915, xvii (in press).

[2] Cited by Butterfield (2). Mohler personally never reported his cases.

[3] Obviously the large mononuclear of the classification presented in this paper.

typical leukemia, pseudoleukemia, or as an anemia with changes in the bone marrow; (2) that mitoses in the blood are pathological and always present in leukemia; (3) that the blood of leukemic fowls contains the virus; and (4) that the disease can be produced by a celi-free Berkefeld filtrate. They conclude (a) that leukemia must be an infectious disease, and (b) that it is to be placed among the diseases due to a filterable virus.

Schridde (10) questioned the infectious etiology of leukemia and claimed that chickens injected with extracts of entirely normal organs present the same changes as Ellermann and Bang reported for leukemia.

Hirschfeld and Jacoby (11, 12) in 1909 observed a typical case of leukemia, and the following year they reported (13) the transmission of the disease from a leukemic animal[4] into the fifth generation.

Burckhardt's claim (14, 15), supported by Friedberger (16), that leukemia could be produced in the fowl by inoculation with pure culture of fowl tubercle bacilli is only of passing interest.

Ellermann (17, 18) in answer to the above objection of Schridde points out that in the latter's experiments only the blood picture was produced, and in the absence of the characteristic organic changes of which Schridde makes no mention, his experiments are of no importance. The injection of emulsion of normal organs Ellermann never found to cause any change in the blood.

Ellermann again reports the successful transmission of the disease with Berkefeld filtrates. Hirschfeld and Jacoby (19) and Burckhardt (15), on the other hand, report unsuccessful results with Berkefeld filtrate.

Ellermann claims to have shown that the leukemic virus can be separated from the virus of tuberculosis by filtration, and that, therefore, the two diseases are distinct. He further claims to be able to separate the spontaneous and transmitted leukemia into the types (a) myeloid and (b) lymphatic. He says that a myeloid type may occur in one generation and a lymphatic in the next, or both types in the same generation and that this is highly suggestive that both forms in man are due to one and the same infective agent.

The Normal Fowl.

The following data of the normal fowl are confined to those portions of the body which are involved in leukemia and have been compiled from a large number of young adult Plymouth Rock hens.

Blood.—The blood is readily obtained from the vein under the wing. From a small needle puncture the blood flows under pressure. It is thick, dark red, and clots quickly.

The number of red blood cells averages 3,000,000 to 4,000,000 per cmm. The number of white blood cells[5] varies between 20,000 to 80,000 per cmm. Actually the proportion of white blood cells to red blood cells varies between 1 to 50 and 1 to 150. The hemoglobin (Sahli) averages 60 to 70 per cent.

[4] This animal was given to them by Ellermann and Bang.

[5] These were determined by the indirect method.

The blood cells stained by Wilson's method may be classified as follows:

Erythrocytes.—1. Normocyte: elliptical disk. Nucleus same shape as cell, deep blue, slightly pyknotic. Cytoplasm yellow and glassy. (Both cell and nucleus are uniform in size, shape, and staining.)

Blood Platelets.—Length of normocyte; width less than that of normocyte. Nucleus round, purple; chromatin diffuse; diameter equal to width of its cell. Cytoplasm pale gray with vacuoles about nucleus, frequently containing small circumscribed red structures. They may vary in size and shape.

Leucocytes.—1. Polymorphonuclear leucocyte with eosinophilic rods: round, diameter about length of normocyte. Nucleus, of two or more lobes, pale blue; chromatin diffuse. Cytoplasm colorless with bright red, spindle-shaped rods.

2. Polymorphonuclear leucocyte with eosinophilic granules: about the same in shape and size. Nucleus of two or more lobes, purple, slightly pyknotic. Cytoplasm faintly blue with dull red granules.

3. Lymphocyte: round, diameter about width of normocyte. Nucleus round, purple; chromatin diffuse. Cytoplasm small in amount, to one side of nucleus, pale blue. Same cell may be slightly larger. Thus a division into small and large lymphocyte may be made.

4. Large mononuclear cell: round or oval, diameter about length of normocyte (at times more or less). Nucleus round, oval, or slightly irregular, and larger; otherwise similar to nucleus of lymphocyte. Cytoplasm abundant, completely surrounds nucleus; pale blue. (A suggestion of fine granules.)

5. Mast cell: about same size and shape. Nucleus round or oval, very pale blue. Cytoplasm abundant, colorless, mostly to one side of nucleus with purple granules; some scattered over nucleus.

Differential count, 300 cells.	*Per cent.*
Polymorphonuclears with eosinophilic rods	29.6
" " " granules	4.3
Lymphocytes	42.3
Large mononuclear cells	19.4
Mast cells	2.2
Unclassified cells	2.2
	100.0

Gross Anatomy.—A fowl of average size and weight (1,760 grams) has abundant subcutaneous fat and large muscles. The inner surface of the skin is slightly yellow.

The cervical lymph glands[6] are sometimes difficult to find. They are present as two chains of six to ten glands, one on each side of the neck, lying in the fat upon the internal jugular vein, and extend-

[6] Kon (4) considers these structures the thymus. He (4) and Ellermann (17, 18) state that the fowl has no lymph glands. Soshestrenski (5) asserts their existence by stating that, in his case, they were not enlarged.

ing from the middle of the neck to the base of the heart. They are elliptical, flat, lobulated bodies, averaging $1 \times 0.5 \times 0.2$ cm. The lobules are pink and separated by narrow septa of fat. No other lymph glands were found in the entire body.

The peritoneal cavity contains an omentum rich in fat, averaging 1 cm. in thickness. It takes its origin from the anterior surface and lower margin of the gizzard, extending down the intestines, etc.

The liver is a bilobed organ, weighing 50 grams, or 2.8 per cent of the body weight. It extends down between the omentum and sternum, the margin of each lobe reaching as far as 3 cm. above the tip of the xiphoid. It is uniformly reddish brown, moderately soft, and friable. The lobulation is usually distinct, at times difficult to see. The cut surface shows small blood vessels in long and cross-section.

The spleen lies just behind the liver. It measures $2 \times 1.5 \times 1$ cm. and weighs 1 gram, or 0.05 per cent of the body weight. It is small, soft, and reddish brown. Beneath the capsule bluish white Malpighian bodies, slightly larger than a pin point, may be indistinctly seen. On section the capsule is very delicate, the trabeculæ are few, but usually definite, containing small blood vessels. The Malpighian bodies at times are prominent. The pulp is not raised above the edge of the capsule. The kidneys weigh 12 grams, or 0.7 per cent of the body weight. They are uniformly reddish brown with a slightly nodular surface.

The bone marrow is mottled, bright red and yellow. It is very soft, at times semifluid. It is always rich in fat.

Histology.—The cervical lymph glands are divided into lobules of parenchyma, separated by fatty tissue. Each lobule is surrounded by a delicate fibrous capsule and has a very fine reticulum, in which the lymphocytes are diffusely scattered. These are small round cells with round, deeply staining, pyknotic nuclei and a narrow rim of pink cytoplasm, usually incompletely surrounding the latter.

Sometimes red blood cells are associated with the lymphocyte; but these are mostly confined to the capillaries. They appear as elongated yellowish pink cells with solid black nuclei, which are seen as rods or dots, according to whether they are in long or cross-

section. Scattered through the lobule are small, sharply outlined, hyaline structures, which Kon (4) considered as corresponding to Hassal's corpuscles of the thymus.

In the liver the lobule is difficult to limit. Periportal spaces are not easily made out. They contain the usual vessels, surrounded by very little acellular fibrous tissue, although lymphocytes may be present diffusely or in small follicles. The liver cells are arranged in trabeculæ, separated by capillaries. All blood vessels and capillaries are filled almost exclusively with red blood cells.

In the spleen the Malpighian bodies are numerous and indistinct. They are composed of the usual lymphocytes surrounding very small arteries. Red blood cells are limited to the pulp where they occur more or less in clusters, although sinuses or inclosures of any kind cannot be definitely demonstrated. The pulp is also diffusely infiltrated with lymphocytes.

The kidneys for all practical purposes have a structure similar to the human kidney.

The bone marrow under low power shows a framework of fatty tissue enclosing nests of marrow cells. Stained with hematoxylin and eosin these may be grouped as follows:

Erythrocytes.—1. Normocyte (a): elongated, varying in shape due to pressure. Nucleus a solid black rod or dot (long or cross-section). Cytoplasm yellowish pink and glassy.

2. Normoblast (b): round; diameter about width of normocyte. Nucleus uniformly black. Cytoplasm pink or faintly blue, and glassy. Frequently a narrow clear zone is seen about the nucleus.

3. Megaloblast (b): the same, larger, about the length of a normocyte. Nucleus slightly pyknotic.

Leucocytes.—1. Polymorphonuclear myelocyte with eosinophilic rods[7] (c): round. Nucleus, two or more solid, black lobes. Cytoplasm colorless with bright red, spindle-shaped rods.

2. Polymorphonuclear myelocyte with eosinophilic granules[7] (b): round; about same size. Nucleus two or more slightly vesicular lobes. Cytoplasm colorless with bright red granules.

3. Mononuclear myelocyte with eosinophilic granules (a): round or oval, varying in size, mostly larger than 1 and 2. Nucleus round, oval, or horseshoe-shaped, eccentric, slightly or very vesicular. Cytoplasm colorless with bright red granules.

[7] These are obviously the polymorphonuclear with eosinophilic rods, the polymorphonuclear with eosinophilic granules, and the large mononuclear cell of the normal blood.

4. Large mononuclear myelocyte[7] (d): round, slightly larger. Nucleus round, very vesicular, one or more nucleoli. Cytoplasm basophilic, moderate in amount.

Reticular Cells.—Branched; nucleus elongated, vesicular; several nucleoli. Cytoplasm pink, giving off delicate fibers to form reticulum.

Lymphocytes, mast cells, platelets, mitoses of red and white cells were not seen. The letters in parenthesis indicate the order of predominance.

Spontaneous Leukemia.

On October 31, 1912, a typical case of leukemia of the fowl was brought to the pathological laboratory. The animal was a Plymouth Rock hen and had just been killed. Nothing is known of its clinical history.

Autopsy Findings.—Blood smears taken from the heart were stained by Wilson's method. A detailed description of the cells of leukemic blood will be reserved for the experimental leukemia where it was possible to study these more carefully. Suffice it here to comment briefly upon the most striking features.

There was an enormous increase in white blood cells, the proportion of white to red cells being 1 to 1.3. The predominating cells were the large mononuclear and the mononuclear myelocyte with eosinophilic granules. The latter is abnormal in the blood, normal in the bone marrow, and when found outside of the bone marrow is typical of leukemia. Lymphocytes and polymorphonuclears were strikingly decreased. Of the latter those with red granules and the mast cell were rare. Mitoses of the large mononuclears were common. The red blood cells appeared poor in hemoglobin, showed anisocytosis, poikilocytosis, and polychromatophilia. These cytoplasmatic changes were usually associated with an increase in the size of the nucleus. Premature red blood cells, normoblasts, and megaloblasts were present in large numbers.

Differential count, 300 cells.	*Per Cent.*
Polymorphonuclears with eosinophilic rods	8
" " " granules	0
Lymphocytes	2
Large mononuclear cells	30
Mast cells	0
Mononuclear myelocytes with eosinophilic granules	52
Unclassified cells	8
	100

Gross Examination of Tissues.—The comb, the featherless area about the eyes, the wattles, the buccal mucous membrane, and the conjunctivæ were very pale. The anterior chamber of the left eye was filled with an old blood clot. A moderately firm, slightly nodular tumor 3.7 × 2 × 1.5 cm. occupied the triangular space on the left side of the head between the angle of the mouth, the ear, and the angle of the lower jaw, extending slightly below the ramus of the latter. Emaciation was extreme. Subcutaneous fat was practically absent. The muscles were greatly atrophied. A second, slightly smaller tumor 1.7 × 1 × 1 cm. was found just inside of the ramus and below the orbit, apparently communicating over the ramus with the first growth and continuous below with a slightly larger third mass.[8]

The cervical lymph glands were somewhat enlarged and showed indistinct lobulation.

In the peritoneal cavity the omental fat was entirely absent and the lower margin of the right and left lobes of the liver extended almost to the pubis.

The liver was enormous. Its surface was extremely mottled. It was reddish brown, and specked with innumerable gray or slightly yellow spots from pin point to a few mm. in diameter, frequently closely packed to form irregular areas, the largest being 1 cm. in diameter. In addition there were scattered gray or slightly yellow nodules averaging about 2 mm. in diameter. The sectioned surface was similar in appearance. The blood vessels were surrounded by a gray zone.

The spleen was enormous. It was about the size of a small hen's egg and diffusely gray.

At the apex of the heart there were several gray spots, pin point to pin head in size.

The kidneys were greatly enlarged and both showed nodules similar to those in the liver.

The bone marrow was gray and poor in fat.

The remaining organs appeared normal.

Microscopical Examination of Tissues.—The normal structure of the cervical lymph glands was changed. The interlobular fat had

[8] A more detailed description and study of these tumors will be reserved for a future communication.

entirely disappeared with approximation of the greatly swollen parenchymatous lobules. Here and there were intra- and interlobular foci of myeloid tissue. These frequently enclosed an artery or vein. There was a more diffuse infiltration of the parenchyma by myeloid cells, filling capillaries, veins, arteries, and in places breaking through the lobular capsule into the interlobular tissue. Of the infiltrating cells two predominated: (1) A large mononuclear, usually round, at times slightly polygonal, with a single round, oval or indented, vesicular, at times multiple nucleus with one or more nucleoli. Its cytoplasm was granular and slightly basophilic. (2) The mononuclear myelocyte with eosinophilic granules, described under normal bone marrow. Mitoses of both cells were common. Normoblasts and megaloblasts were also present.

In the liver the process was most extreme. Very little liver tissue remained. Everywhere were closely packed masses of myeloid cells, in which the liver trabeculæ had completely disappeared. These masses of cells frequently surrounded blood vessels, both arteries and veins, infiltrating the walls of the latter and filling the lumina of both. Within the vessels, the white blood cells were present in about equal proportions with the normocytes. These cells also occurred everywhere between the liver columns, spreading them apart. It was usually difficult to demonstrate the capillary wall. The hepatic cells in these areas had not suffered so much. Some of the circumscribed infiltrations showed a coarse sclerosis. The infiltrating cells were the same as in the cervical lymph glands. A few polymorphonuclear cells of both types were present.

The pulp of the spleen was diffusely infiltrated with closely packed leukemic cells, crowding the reticulum and distending blood vessels, separating and compressing the Malpighian bodies. Mononuclear myelocytes with eosinophilic granules although present were somewhat scarce. The large mononuclear with mitoses was the common cell.

The bone marrow was greatly changed. It consisted of closely packed white marrow cells, with complete atrophy of the fat, and a great rarity of normocytes. One or two small areas of sclerosis were present. The marrow cells, both red and white, answered the same description as normal bone marrow, with the exception of the

large mononuclear myelocyte, which showed considerable variation in its nucleus. This was single or multiple, round, oval, or horse-shoe-shaped. The order of predominance had changed. The large mononuclear myelocyte was present in far greater numbers than any other cell, and showed extensive mitoses. Normoblasts and megaloblasts with mitoses followed next in frequency, then normocytes. Polymorphonuclear myelocytes, both with eosinophilic rods and granules, had disappeared entirely. The mononuclear myelocyte with eosinophilic granules could not be demonstrated with certainty. As in the normal marrow, lymphocytes, mast cells, and platelets were seen.

In the remaining organs, the blood vessels were filled with the characteristic blood. Cell infiltrations, both diffuse and focal, occurred in the heart, lungs, and kidneys.

Summary. Blood.—(a) Although a total count was not made, a great increase in the total number of white blood cells was evident from the appearance of the blood smears, and blood vessels in sections. The actual proportion of white to red cells (1 to 1.3) substantiated this fact. The differential count showed a marked increase of the large mononuclear cell at the expense of the other white blood cells of the normal blood. In addition, a true myelocyte appeared in the circulation. Mitoses of the large mononuclear in the circulation were common. (b) There was a corresponding decrease in the total number of red blood cells. These were poor in hemoglobin and showed variation in size and shape, basophilic staining of the cytoplasm, and swelling of their nuclei. Premature red cells also occurred.

Organs.—(a) Many organs contained diffuse or circumscribed infiltrations of myeloid cells. The large mononuclear and mononuclear myelocyte with eosinophilic granules predominated in these infiltrations although the other cells of the normal bone marrow were also present. This myelosis involved especially the liver, spleen, kidneys, and bone marrow, resulting in an extensive enlargement of the first three organs. Almost all the remaining organs show infiltration, but to a less degree. (b) The proportion of white to red cells was greatly increased in the blood vessels. The predominating cells were the same as in the infiltration.

Conclusion.—If we consider that the cells characteristic of both the leukemic infiltration and blood are the same, and that under normal conditions the mononuclear monocyte with eosinophilic granules occurs only in the bone marrow and the large mononuclear only in the marrow and blood, it is evident from the summarized facts that the above case must be considered a typical case of myeloid leukemia.

Experimental Leukemia.

From the above animal, the disease was transmitted into the 5th generation. A total of 105 animals was used in conducting many different kinds of experiments. Of this number, 22 in all developed leukemia. In 4 additional animals, a definite diagnosis could not be established, although they were highly suggestive.

This paper will be confined to a report of the simple transmission of the disease by injection of an organic emulsion.

Five series of experiments were conducted, each consisting of 5, 10, or 15 fowls, with 20 to 40 per cent of positive animals. Of the total number of 40 chickens injected, 13 developed leukemia; *i. e.*, 32.5 per cent. In addition, a definite diagnosis could not be made in 3, which were very suspicious.

For transmission pieces of liver, sometimes also spleen, were thoroughly macerated in 0.9 per cent salt solution and filtered through a single layer of fine linen or a small amount of raw cotton. This emulsion could be used for intraperitoneal injection. When used intravenously, however, instant death resulted, and it was necessary to filter it also through two layers of filter paper with the aid of a suction pump for successful intravenous inoculations. This filtrate still contained blood and parenchymatous cells. A 15 per cent emulsion gave the best results. 10 cc. of the emulsion were injected either intravenously or intraperitoneally. Both methods were sometimes combined when a total of 20 cc. was administered. The vein under the wing was selected for injection.

Animals used for transmission were young adult hens of the same breed as the spontaneous case and exemplified by the normal control.

Clinical History.—The incubation period is usually from 5 to 6 weeks, with a maximum of 16 weeks.

The onset is as a rule abrupt, beginning with a slight but progressive, at times a sudden and intense, pallor, affecting comb, featherless area about eyes, and wattles. This is almost invariably associated with jaundice, which is likewise progressive and usually reaches an extreme grade. A characteristic yellowish pink color results which gives the fowl a ghastly appearance. Comb, etc., may be surprisingly red or extremely pale without jaundice. The animal emaciates rapidly and to an extreme degree. The actual loss in weight during the disease is striking. No. 28 at the onset of the disease weighed 1,485 gm. The duration of the disease was 3 weeks and 4 days. Weight at death was 897 gm., a loss, therefore, of 588 gm. in 25 days, or at the rate of 24 gm. per day.

At first the fowl acts normally, or only slightly ill. In a few days, however, it is very sick, stops eating and drinking, and stands about with head retracted, eyes closed, and tail drooping. It prefers to assume a squatting position. If made to move, it does so very slowly and carefully. Weakness becomes more and more marked, until on the last day it lies on the floor, wings drooping, eyes closed, and is at times dyspneic. Fever has never been noticed.

After the animal is observed to be sick the illness lasts from 1 to 2 weeks. One case, exceptionally acute, lasted only 33 hours. The longest course was 4 weeks.

Only in one case, out of 23 leukemic animals, did a spontaneous cure result.

Blood.—With the onset of anemia, the vein under the wing collapses and the blood undergoes a change. It soon flows with the greatest ease, is pale yellow and watery, and shows no tendency to clot. The animal apparently would bleed to death from a pin point wound if hemorrhage were not artificially arrested.

In most cases there is a progressive and extreme decrease in the total number of red blood cells. At the onset the count may be but slightly below the normal, 2,224,000, while just before death it at times reaches 630,000 per cmm. In one case the count remained normal. The white blood cells are invariably high, 131,200 to 210,000 per cmm. The ratio of white blood cells to red blood cells varies between 1 to 3 and 1 to 9.

The hemoglobin usually falls steadily and reaches a very low point. From slightly below normal, 40 to 50 per cent at onset, it frequently drops to 10 to 15 per cent just before death. In one case there was no change at all.

The morphology of the leukemic blood is exceptionally interesting. In addition to the enormous increase in the number of white cells, all the cellular elements show marked changes and many new forms make their appearance.

The blood cells stained by Wilson's method may be classified as follows:

Erythrocytes.—1. Normocyte: (a) normal, except for variation in the amount of hemoglobin; (b) showing anisocytosis, poikilocytosis, and polychromatophilia, mostly associated with a swelling of the nucleus and a separating of its chromatin.

2. Normoblast: round; diameter less than length of normal normocyte. Nucleus of the same color, or slightly purple, with more scattered chromatin than nucleus of normal normocyte. Cytoplasm greenish blue and glassy, frequently with a clear zone about the nucleus.

3. Megaloblast: the same, except that the diameter is equal to or greater than the length of the normal normocyte.

4. Mitotic cells: all stages, from monaster to complete division of nucleus. (a) Round or elliptical; diameter about the length of the normal normocyte. Dense, deep blue chromosomes, massed in center of cell. Cytoplasm greenish blue and glassy. (b) The same, with two masses of chromosomes in opposite extremes of cell. (c) The same (at times with a slight constriction in the middle of the cell) with irregular dense, deep blue or slightly purple chromatin masses in place of individual chromosomes. (d) The same, with two nuclei, similar in appearance to those of the normoblast. (e) The same as the normal normocyte with two nuclei, similar in appearance to those of the normoblast, but only slightly larger than nucleus of normal normocyte. a, b, c, and d may show polychromatophilic cytoplasm.

Blood Platelets.—1. The same as normal, except that both cell and nucleus are larger.

2. Larger than normal with two nuclei.

Leucocytes.—1. Polymorphonuclear leucocyte with eosinophilic rods: the same as in normal blood, at times possibly a little smaller.

2. Polymorphonuclear leucocyte with eosinophilic granules: the same as in normal blood, at times possibly a little smaller.

3. Lymphocyte: the same as in normal blood.

4. Large mononuclear cell: (a) as described in normal blood with a little less cytoplasm. (b) The same size to one-half times larger than (a), with both nucleus and cytoplasm paler. (a) and (b) cannot be separated absolutely. Every gradation. (c) Mitoses: all stages from monaster to complete separation of nucleus: (a′) elliptical; diameter about 1.5 times the length of the normal normocyte. Dense, purple chromosomes centrally massed. Cytoplasm pale bluish gray, granular. (b′) The same, with two masses of chromosomes in opposite extremes of the cell. (c′) The same, with two purple nuclei. (d′) The same, with three purple nuclei.

5. Mast cell: as described in normal blood.

6. Mononuclear myelocyte with eosinophilic granules: round or slightly elliptical, diameter about length of normal normocyte or longer. Nucleus oval, pale blue with dense chromatin, eccentric. Cytoplasm colorless with small and large bright red granules; some scattered over nucleus.

Cytoplasmatic masses without nuclei: round or slightly oval; diameter varies, usually about width of normocyte. No nucleus. Cytoplasm grayish blue, sometimes with vacuoles.

The above is the blood picture common to all leukemic animals. The order of predominance of the white cells is typical of leukemia, differing from the normal, but agreeing with that of the spontaneous case. The large mononuclear is present in by far the greatest number. The other cells of the normal blood are decreased. Polymorphonuclears with eosinophilic granules and mast cells are very scarce. The mononuclear myelocyte with eosinophilic granules, although not as common as in the spontaneous case, can usually be demonstrated in every leukemic animal. The platelets are greatly increased in number.

Differential count, 300 cells.	*Per cent.*
Polymorphonuclears with eosinophilic rods	6
" " " granules	0
Lymphocytes	4
Large mononuclear cells	86
Mast cells	1
Mononuclear myelocytes with eosinophilic granules	1
Unclassified cells	2
	100

Autopsy Findings. Gross and Microscopical.—The weight of the animal in every case was far below its weight before injection.[9] The two lowest weights, at autopsy, were 675 and 897 grams.

The jaundice, at times, involved the skin of the entire body.[10] The conjunctivæ and buccal mucous membrane were always very pale. Emaciation was extreme. There was a great scarcity or entire absence of subcutaneous fat. Muscular atrophy was usually marked; at times practically only the skeleton remained.

The cervical lymph glands, macroscopically, were, as a rule, uninvolved. In two cases, they were definitely enlarged, in the one very much so. They measured $1.5 \times 8 \times 4$ cm. and $2 \times 1 \times 5$ cm., respectively. They appeared uniformly gray with absence of lobulation due to parenchymatous swelling and atrophy of interlobular fat. In a third case they were of normal size and appearance, but showed several gray nodules 1 mm. in diameter. Microscopically, those glands which appeared normal in gross were usually free

[9] Animals which remained negative invariably gained weight.

[10] Probably this was more frequent but not recognized, except when very grave, because of the normal yellow tint of the skin.

of myeloid infiltration, although their blood vessels contained leukemic blood. The diffusely enlarged glands showed a marked swelling of their parenchyma with complete atrophy of the interlobular fat. Myeloid cells, both the large mononuclear and the mononuclear myelocyte with eosinophilic granules, were scattered through the lobules. Foci, entirely of the first, or exclusively of the second, were localized principally in the interlobular connective tissue. They were rich in mitotic figures. The gray nodules proved to be a mass of proliferating myeloid tissue.

In the peritoneal cavity, the omental fat was greatly decreased. At best, it was present only in moderate amount. As a rule, it was replaced by a thin yellow membrane. Ascites occurred in 6 out of 13 cases, at times in sufficient amount to distend the abdomen. It was always associated with a serofibrinous mass which covered the liver and most of the other abdominal viscera and the outer surface of the pericardium. With the exception of Nos. 31 and 70 every animal of the thirteen had an enlarged liver. The margins of the right and left lobes of the smallest of the enlarged livers were respectively 1 and 3 cm. above the xiphoid, while the margins of the largest liver extended 3.5 and 3 cm. respectively below the xiphoid. In fact, the latter organ *in situ* was so enormous that it filled the entire peritoneal cavity and was the only viscus visible on opening the abdomen.

The liver was frequently enormous. Its weight ranged from 80 to 265 grams; *i. e.*, over five times normal, or 6.2 to 10.3 per cent of body weight, an increase of 3.6 times normal.

The external appearance of the liver was usually very characteristic. It frequently appeared diffusely gray, due to very closely packed subcapsular spots, pin point to 2 mm. in diameter. These were usually more scattered and translucent, at times slightly yellow and opaque. They were often fused to form larger areas with a diameter of 0.5 to 1 cm., or they were arranged in a delicate gray network. In addition, numerous gray or slightly yellow nodules ranging from 0.2 to 0.5 cm. in diameter were often present. The whole gave the surface an extremely mottled appearance. Lobulation at times was visible, usually indistinct. The liver was either slightly firm or friable. The sectioned surface appeared very similar

to the external surface. Dots and lines often formed gray borders along the blood vessels. The latter also occupied the centers of some of the larger, circumscribed gray areas. The perivascular connective tissue in rare instances was bile-stained. Microscopically, there was usually a diffuse infiltration of myeloid cells. They were closely packed within and without the intralobular capillaries. The liver trabeculæ showed fatty degeneration and atrophy. The large mononuclear, rich in mitotic figures, was by far the predominating cell. The mononuclear myelocyte with eosinophilic granules was very scarce. Scattered through the section were foci of large mononuclears, or of mononuclear myelocytes with eosinophilic granules. Some foci contained both types of cells. They abounded in mitotic figures. The liver cells in these foci had entirely disappeared. The small, gray nodules consisted of circumscribed masses of myeloid tissue, usually occupying the periportal spaces and composed of a central portion of large mononuclears surrounded by the mononuclear myelocyte with eosinophilic granules. In the blood vessels and capillaries the large mononuclear at times seemed to exceed greatly the normocyte in number.

The spleen with the exception of Nos. 31 and 70 was, in every case, enlarged, usually extremely. The smallest of the enlarged organs measured $3 \times 2 \times 1.7$ cm., the largest $4 \times 3 \times 2.3$ cm. The latter was just twice normal in every dimension. The lowest weight was 6 grams, the highest 18 grams; *i. e.,* 18 times normal, or 0.6 to 1.6 per cent of body weight; *i. e.,* 32 times normal.

The surface of the spleen was sometimes normal. More frequently, it was studded with scattered gray or slightly yellow spots and nodules. The first was from 1 to 3 mm., and the largest of the second from 0.5 to 1 in diameter. Again, the organ appeared diffusely gray. On section it was usually similar to the surface. When diffusely gray, the pulp was somewhat granular and in excess. Microscopically the entire pulp was often diffusely infiltrated with the large mononuclear cell separating and compressing the Malpighian bodies. In addition, circumscribed closely packed masses of pure large mononuclears or mononuclear myelocytes with eosinophilic granules were scattered through the section. Occasionally, the myelosis was only present in foci, with practically no disturbance

of normal splenic structure. Mitoses were abundant wherever the large mononuclear occurred.

The kidneys of 5 of the 13 cases were enlarged. They weighed from 12 to 26 grams; or 0.8 to 1.4 per cent of the body weight.

The surface and section of the kidney were entirely normal, but usually there were below its capsule scattered, gray, pin point dots, delicate lines, or even one or two nodules, the largest 0.5 cm. in diameter. Again, they appeared diffusely gray. Microscopically, the kidneys always showed more or less distention of their blood vessels and intertubular capillaries with the characteristic leukemic blood in which white blood cells, at times, even seem to exceed the normocytes, and in which the large mononuclears predominated. When capillary distention was moderate, the tubules appeared normal, but at times they were so enormously overfilled as to cause extreme atrophy and degeneration of the renal epithelium, resulting frequently in circumscribed areas devoid of any parenchyma. These areas were more pronounced about the larger blood vessels. The walls and the perivascular tissue of some of the vessels were infiltrated with actively generating myeloid cells, especially the large mononuclear.

The bone marrow was always involved, usually more or less characteristically. It was increased in amount, moderately soft, and red, with countless gray dots to slightly larger areas; or it was diffusely gray. Microscopically, the normal structure of the marrow was greatly changed. The fatty tissue had completely disappeared and the marrow cells formed a solid mass. The description of the red and white marrow cells agreed with that given under normal marrow. Possibly the large mononuclear was more commonly polygonal, due to pressure. Mitoses of both the large mononuclear and the erythrocytes were common. The order of predominance was changed from normal. The large mononuclear myelocyte was by far the most abundant; the normoblast, the megaloblast, and the mononuclear myelocyte with eosinophilic granules followed in about equal numbers; the normocyte was least numerous. Both types of polymorphonuclear as well as the lymphocyte, mast cells, and platelet were not seen at all. Mitoses of the mononuclear myelocyte with eosinophilic granules were not demonstrated.

In the remaining organs and tissues the blood vessels were filled with the characteristic blood; and in many, infiltrations of myeloid cells occurred in moderate degree.

Summary.—The essential points deduced from the simple transmission of leukemia by injection of an organic emulsion may be stated as follows:

Clinical History.—After an incubation period, usually from 5 to 6 weeks, the fowl suddenly becomes pale, jaundiced, emaciates rapidly, loses weight, and shows signs of extreme weakness, followed almost without exception by death in 1 to 2 weeks.

Blood.—(a) The total number of white blood cells is greatly increased, resulting in a proportion of one white to three red blood cells. The differential count showed a marked increase and predominance of the large mononuclear over the other white cells of the normal blood, which have decreased. The mononuclear myelocyte with eosinophilic granules is present in more or less numbers in practically every case. Besides the normal appearing large mononuclear, one sees many larger pale forms. In these cells, typical and atypical, mitoses in all stages are common. (b) The total number of red blood cells is correspondingly decreased, with a marked fall in hemoglobin. They present varieties in size, shape, and staining, associated with swelling of the nucleus. Normoblasts and megaloblasts, with mitoses in all stages, make their appearance. (c) There is an increase in the number of blood platelets, associated with an increase, both in size of the cell and its nucleus. The cells frequently contain more than one nucleus. (d) The clotting power of blood is greatly decreased.

Organs.—(a) A diffuse or focal infiltration of marrow cells occurs in many organs. The large mononuclear and mononuclear myelocyte with eosinophilic granules predominate and may occur separately or together in the same nodule. Extensive mitoses occur in both these cell types. This myelosis affects more particularly the liver, spleen, kidneys, and bone marrow, causing a great increase in the size of the first three organs. Rarely the cervical lymph glands are also very much enlarged. Most of the remaining organs and neighboring tissues may contain infiltrations, but not so extensive. (b) The proportion of white to red blood cells is greatly increased

in the blood vessels. The same cells predominate as in the infiltration. (c) The atrophic and degenerative changes of the parenchyma and of the adipose tissue are marked; ascites occurs.

Conclusion.—From the above experimental study the following conclusions may be drawn: The injection of an organic emulsion causes a picture of myeloid leukemia in every respect similar to spontaneous leukemia as it occurs in the fowl. The clinical picture and changes produced in blood and organs are analogous to those which occur in human leukemia.

CONCLUSIONS.

1. The spontaneous occurrence of myeloid leukemia of the fowl is confirmed.

2. Myeloid leukemia of the fowl is transmissible by intravenous or intraperitoneal injection of an organic emulsion.

The latter is in confirmation of the work of Ellermann and Bang (6, 7), who first successfully transmitted the disease. They were followed by Hirschfeld and Jacoby (13), whose successful transmissions, however, seem to be limited to a strain which had its origin in a fowl presented to them by Ellermann and Bang. Burckhardt (14, 15) likewise transmitted the disease, but here again the stock animal came from Hirschfeld and Jacoby and therefore indirectly from Ellermann and Bang.

The transmission reported above is of special interest, because it originated in an animal absolutely unrelated to that of the previous investigators.

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